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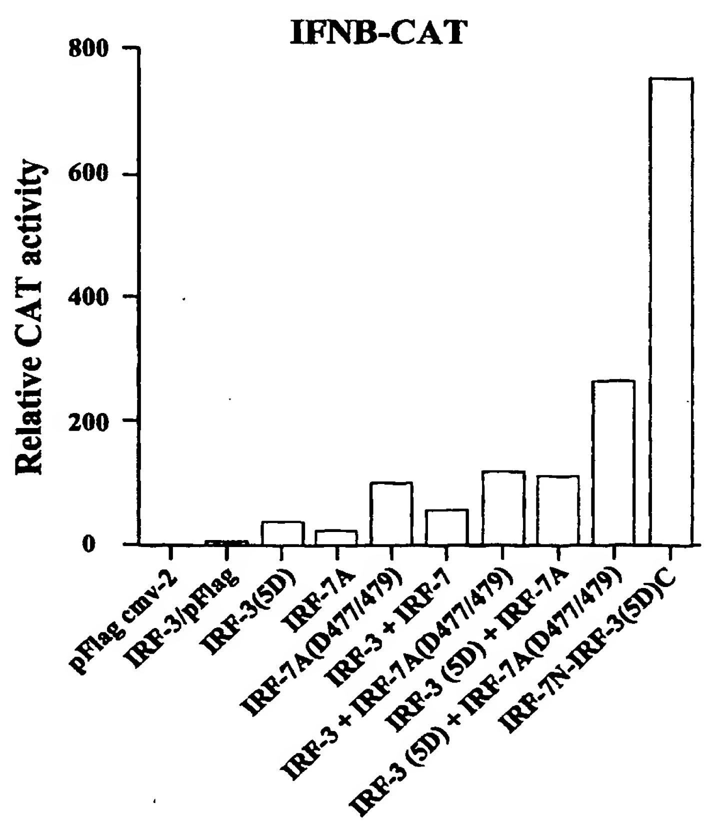
(54) Title: HIGHLY ACTIVE FORMS OF INTERFERON REGULATORY FACTOR PROTEINS

(57) Abstract

The present invention relates to IRF proteins that have been modified in the carboxy–terminus domain (transactivation domain) by modification of serine and/or threonine sites. Modification may be achieved by phosphorylation of serine and/or threonine, or by replacement of serine and/or threonine residues with residues having acidic side–chains, preferably carboxylic acid–containing side–chains, such as aspartic acid or glutamic acid residues. Such modified proteins may be mutants of IRF–3 and IRF–7, including chimeric proteins having portions of both IRF–3 and IRF–7, and post–translationally modified (phosphorylated) IRF–3 protein, the phosphorylation being induced by Sendai virus infection. More specifically, the present invention provides a modified interferon regulatory factor (IRF) protein, the protein comprising at least one modified serine or threonine phosphoacceptor site in the carboxy–terminus domain, preferably wherein cytokine gene activation by the modified IRF is increased relative to cytokine gene activation by a corresponding wild type IRF protein. The invention also provides for pharmaceutical compositions containing IRF protein, and uses of the protein, nucleotide sequence encoding it, and pharmaceutical compositions containing it.



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HIGHLY ACTIVE FORMS OF INTERFERON REGULATORY FACTOR PROTEINS

BACKGROUND OF THE INVENTION

Interferons (IFNs) are a large family of multifunctional secreted proteins involved in antiviral defence, cell growth regulation and immune activation (63). Virus infection induces the transcription and synthesis of multiple IFN genes (33,52,63); newly synthesized IFN interacts with neighbouring cells through cell surface receptors and the JAK-STAT signalling pathway, resulting in the induction of over 30 new cellular proteins that mediate the diverse functions of the IFNs (17,35,39,58). Among the many virus- and IFN-inducible proteins are the growing family of IRF transcription factors, the Interferon Regulatory Factors (IRFs).

IRF-1 and IRF-2 are the best characterized members of this family, originally identified by studies of the transcriptional regulation of the human IFN-β gene (22,23,30,47). Their discovery preceded the recent expansion of this group of IFN-responsive proteins which now include seven other members: IRF-3, IRF-4/Pip/ICSAT, IRF-5, IRF-6, IRF-7, ISGF3γ/p48 and ICSBP (48). Structurally, the Myb oncoproteins share homology with the IRF family, although its relationship to the IFN system is unclear (62). Recent evidence also demonstrates the presence of virally encoded analogue of cellular IRFs - vIRF in the genome of human herpes virus 8 (HHV8) (55).

The presence of IRF-like binding sites in the promoter region of the IFNA and IFNB genes implicated the IRF factors as essential mediators of the induction of IFN genes. The original results of Harada et al. (30,32) indicated that IFN gene induction was activated by IRF-1, while the related IRF-2 factor suppressed IFN expression. However, the essential role of IRF-1 and IRF-2 in the regulation of IFNA and IFNB gene expression has become controversial with the observation that mice containing homozygous deletion of IRF-1 or IRF-2, or fibroblasts derived from these mice, induced IFNA and IFNB gene

expression after virus infection to the same level as the wild-type mice or cells (44).

On the other hand, IRF-1 was shown to have an important role in the antiviral effects of IFNs (44,54). IRF-1 binds to the ISRE element present in many IFN-inducible gene promoters and activates expression of some of these genes (54). However, activation of ISG genes by IFNA and IFNB was shown to be mediated generally by the multiprotein ISGF3 complex (31,36,38). The binding of this complex to DNA is mediated by another member of the IRF family, ISGF3γ/p48, which in IFN-treated cells interacts with phosphorylated STAT1 and STAT2 transcription factors forming the heterotrimeric complex ISGF3 (8,39,62). The homozygous deletion of p48 in mice abolished the sensitivity of these mice to the antiviral effects of IFNs, and virus-infected macrophages from p48-/- mice showed an impaired induction of IFNA and IFNB genes (31).

Several other members of the IRF family have been identified. The ICSBP gene is expressed exclusively in the cells of the immune system (18,64) and its expression can be enhanced by IFNγ. ICSBP was shown to form a complex with IRF-1 and inhibit the transactivating activity of IRF-1 (9,59). The homozygous deletion of ICSBP in mice leads to defects in myeloid cell lineage development and chronic myelogous leukemia (34). Another lymphoid specific Pip/LSIRF/IRF-4 was identified (19,43,66) that interacts with phosphorylated PU.1, a member of the Ets family of transcription factors (15). The Pip/PU.1 heterodimer can bind to the immunoglobulin light chain enhancer and function as a B cell specific transcriptional activator. Expression of Pip/LSIRF was induced by antigenic stimulation but not by IFN, and Pip/LSIRF/IRF-4 -/- mice failed to develop mature T and B cells (46). A novel member of the IRF family was recently identified by its ability to bind to an ISRE-like element in the promoter region of the Qp gene of EBV (69).

Another unique member of the human IRF family, IRF-3 was characterized recently (2). The IRF-3 gene encodes a 55-kDa protein which is expressed constitutively in all tissues. IRF-3 was originally identified as a member of the

IRF family based on homology with other IRF family members and on binding to the ISRE of the ISG15 promoter. The relative levels of IRF-3 mRNA do not change in virus-infected or IFN-treated cells. Recombinant IRF-3 binds to the ISRE element of the IFN-induced gene ISG-15 and stimulates this promoter in transient expression assays. In previous studies, it has been shown that IRF-3 binds to the IE and PRDIII regions of the IFNA and IFNB promoters respectively, but has different effects on their transcriptional activity (56). While the induction of the IFNA4 promoter activated by IRF-1 or virus infection was inhibited in the presence of IRF-3, the fusion protein containing the IRF-3 DNA binding domain and the RelA(p65) transactivation domain effectively activated both IFNA and IFNB promoters. In contrast, co-expression of IRF-3 and RelA plasmids transactivated the IFNB gene promoter, but not the promoter of the IFNA4 gene (56).

Most of the IRF family members so far identified appear to have specific and critical functions in lymphoid cells and/or their action is related to the signalling pathway induced by IFN or viruses. Interestingly, there is recent evidence indicating that the IRF(s) may also play a role in the transcriptional activation of viral promoters. The Qp promoter region of the EBV-encoded gene EBNA-1 contains an ISRE-like element that is responsive to the IRF-1 and IRF-2 as well as to IFN-α. Using a yeast one-hybrid screen technique, a new factor was recently isolated that binds specifically to the Qp ISRE. The amino acid sequence of this protein is identical to the IRF-7 protein present in the Genbank database ((69); accession number U73036). By homology search of the HGF ETS cDNA library the Pitha group has also identified a novel IRF whose sequence is identical to that of IRF-7. At the amino acid level, IRF-7 shows highest homology to IRF-3. Several open reading frames (ORFs) of IRF-7 have been identified. Pagano's group found three shorter ORFs, listed in the database as IRF-7A, B and C ((69), accession nos. U53830, U53831 and U53832, respectively). A new IRF-7 isoform, IRF-7H, was recently identified by Pitha's group ((70), accession number AF076494).

In vitro translated IRF-7 encodes a protein of 68 kDa (69, 72). Interestingly, while *in vitro* translated IRF-7 protein binds effectively to the Qp ISRE, it doesn't seem to affect transcription of Qp-driven reporter constructs in a transient transcription assay (72). In contrast to IRF-3, IRF-7 expression is not generally constitutive but can be effectively induced by IFN-α in fibroblast cells, B-cells and other cells of lymphoid origin (70, 71). Like IRF-3, in uninfected cells, IRF-3 is present mainly in the cytoplasm, virus infection induced phosphorylation of IRF-7, resulting in cytoplasmic to nuclear translocation of phosphorylated IRF-7 and activated gene transcription (70, 71). Recent studies indicate that virus-stimulated phosphorylation of IRF-3 results in the activation of IFNα4 and IFNβ gene transcription in murine cells. Once produced and secreted from the infected cell, IFNα4 and IFNβ subsequently feed back on cells through the IFN receptor, stimulate the Jak-STAT pathway and lead to the IFN-responsive activation of another member of the IRF family - IRF-7; up-regulation of IRF-7 production then mediates the induction of non-IFNα4 gene expression (71).

SUMMARY OF THE INVENTION

The present invention relates to IRF proteins that have been modified in the carboxy-terminus domain (transactivation domain)by modification of serine and/or threonine sites. Modification may be achieved by phosphorylation of serine and/or threonine, or by replacement of serine and/or threonine residues with residues having acidic side-chains, preferably carboxylic acid-containing side-chains, such as aspartic acid or glutamic acid residues. Such modified proteins may be mutants of IRF-3 and IRF-7, including chimeric proteins having portions of both IRF-3 and IRF-7, and post-translationally modified (phosphorylated) IRF-3 protein, the phosphorylation being induced by Sendai virus infection.

More specifically, the present invention provides a modified interferon regulatory factor (IRF) protein, the protein comprising at least one modified serine or threonine

phosphoacceptor site in the carboxy-terminus domain, preferably wherein cytokine gene activation by the modified IRF is increased relative to cytokine gene activation by a corresponding wild type IRF protein.

The present invention also provides a pharmaceutical composition comprising an effective amount of the interferon regulatory factor (IRF) protein according to the invention, together with a pharmaceutically acceptable carrier, for the treatment of a viral infection, for example, an influenza infection, a herpes infection or an HIV infection.

The present invention further provides use of the interferon regulatory factor (IRF) protein according to the invention to activate a cytokine gene, preferably wherein the cytokine gene is an interferon gene or a chemokine gene.

DESCRIPTION OF THE FIGURES

Figure 1. Sendai virus infection induces IRF-3 degradation.

IRF-3 expression plasmid CMVBL-IRF3 (lanes 1 and 2) or CMVBL vector alone (lanes 3 and 4), both at 5 μg were transiently transfected into 293 cells by the calcium phosphate method. At 24h post transfection, cells were infected with Sendai virus for 16h (lanes 2 and 4) or left uninfected (lanes 1 and 3). Whole cell extracts (20 μg) were prepared and analyzed by immunoblotting with anti-IRF-3 antibody.

Figure 2. Sendai virus induced phosphorylation and degradation of IRF-3 protein.

A) rtTA-IRF-3 cells, selected as described in the Example, were induced to express IRF-3 by doxycycline treatment for 24h. At 24h after Dox addition, cells were infected with Sendai virus for 4, 8, 12, 16, 20, or 24h (lanes 2-7) or were left uninfected (lane 1). IRF-3 protein was detected in whole cell extracts (10 μg) by immunoblot. Two forms of IRF-3 were detected, designated as form I and form II.

B) At 24h post Dox induction, rtTA-IRF-3 cells were infected with Sendai virus for 16 hours (lanes 4-8) or were left uninfected (lanes 1-3). Whole cell extracts from untreated

cells (20 μg) or Sendai virus infected cells (60 μg) were incubated with 0.3 units of potato acidic phosphatase (PPA, lanes 2, 3, 7 and 8) or 5 units of calf intestinal alkaline phosphatase (CIP, lanes 4 and 5) in the absence (lanes 1, 2, 4, 6 and 7) or presence of phosphatase inhibitors (lanes 3, 5 and 8). Phosphorylated IRF-3 protein appears as a distinct band in immunoblots, migrating more slowly than IRF-3 forms I and II.

Figure 3. Analysis of IRF-3 deletion mutants in Sendai virus induced phosphorylation.

(A) Schematic representation of four IRF-3 deletions. Thick solid lines and thin dashed lines indicate included and excluded sequences, respectively. The N-terminal IRF homology domain, the nuclear export signal (NES) and C-terminal IRF association domain are indicated.

(B) Expression plasmids (5 μg each) encoding wild type and deletion mutants of IRF-3 (as indicated above the lanes) were transiently transfected into 293 cells; at 24h post transfection, cells were infected with Sendai virus for 16h (lanes 2, 4, 6, 8, and 10) or left uninfected (lanes 1, 3, 5, 7, and 9). Whole cell extracts (20 μg) were prepared from infected and control cells and analyzed by immunoblotting for IRF-3 forms I and II and for the presence of phosphorylated IRF-3 (P-IRF-3) with anti-IRF-3 antibody.

Figure 4. Analysis of IRF-3 point mutations in Sendai virus induced phosphorylation.

(A) Schematic representation of IRF-3 point mutations. Thick solid lines and thin dashed lines indicate included and excluded sequences, respectively. The N-terminal IRF homology domain, the Nes element and C-terminal IRF association domain are indicated. Amino acids residues from 382 to 414 and from 141 to 147 are shown. The amino acids targeted for alanine or aspartic acid substitution are shown in large print. The point mutations are indicated below the sequence: (2A: S396A/S398A; 3A: S402A/T404A/S405A; 5A: S396A/S398A/S402A/T404A/S405A); 5D S396D/S398D/S402D/T404D/S405D; J2A: S385A/S386A; NES: S145A/S146A).

(B) Expression plasmids (5 μg each) encoding wild type and

point mutants of IRF-3 (as indicated above the lanes) were transiently transfected into 293 cells; at 24h post transfection, cells were infected with Sendai virus for 16h (lanes 2, 4, 6, 8, 10, 12, 14, 16 and 18) or left uninfected (lanes 1, 3, 5, 7, 9, 11, 13, 15 and 17). Whole cell extracts (20 μg) were prepared from infected and control cells and analyzed by immunoblotting for IRF-3 forms I and II and for the presence of phosphorylated IRF-3 (P-IRF-3) with anti-IRF-3 antibody.

Figure 5. Virus dependent cytoplasmic-nuclear translocation of IRF-3.

The subcellular localization of the GFP-IRF-3 (A and B), GFP-IRF-3(5A) (C and D), GFP-IRF-3(5D) (E and F) and GFP-IRF-3(NES) (G and H) was analyzed in uninfected (A, C, E, and G) and Sendai virus infected COS-7 cells at 16h after infection. GFP fluorescence was analyzed in living cells with a Leica fluorescence microscope using 40x objective.

Figure 6. Transactivation of PRDI/PRDIII and ISRE containing promoters by IRF-3.

293 cells were transfected with IFNβ-CAT (A and B) or ISG15-CAT (C) reporter plasmids and the various expression plasmids as indicated below the bar graph. CAT activity was analyzed at 48h post-transfection with 100 μg (IFNβ-CAT) or 10 μg (ISG15-CAT) of total protein extract for 1-2h at 37°C. Relative CAT activity was measured as fold activation (relative to the basal level of reporter gene in the presence of CMV-Bl vector alone after normalization with co-transfected β-Gal activity); the values represent the average of three experiments with variability shown in the error bar.

Figure 7. IRF-3 inducible expression of RANTES gene.

(A) Stimulation of RANTES gene transcription in virus-infected and IRF-3(5D)-expressing cells. The rtTA, IRF-3 and IRF-3(5D) cells were cultured in the presence or absence of Dox as indicated. After 30 hours, cells were either left untreated, infected with Sendai virus (80HAU/ml) for 16 hours, or treated with IFN-α/β (100 IU/ml). The neutralizing antibody for type I IFN (Sigma) was added at the time of Dox addition.

Total RNA was isolated from each sample and analyzed by RPA using the hCK5 kit (Pharmingen).

(B) Repression of virus-induced RANTES gene transcription by a dominant-negative form of IRF-3. The rtTA- and IRF-3(ΔN)-expressing cells were either left untrated or infected with Sendai virus (80 HAU/ml) for 16 hours. Total RNA was isolated from each sample and analyzed by RPA.

(C) The kinetics of RANTES expression induced by IRF-3 (5D). Total RNA from IRF-3(5D)-expressing cells was isolated from each sample after Dox addition and analyzed by RPA.

(D) Cell culture supernatants were analyzed for the presence of RANTES protein by an ELISA performed as specified by the manufacturer (Biosource International).

Figure 8. Stabilization of IRF-3 by proteasome inhibitors.

IRF-3 ΔN (Δ9-133) (B) or IRF-3 ΔN2A (C) expression plasmids were transiently transfected into 293 cells; at 24h post transfection, cells were infected with Sendai virus and treated for 12h with calpain inhibitor I (100 μM, lanes 2 and 5) or MG132 proteasome inhibitor (40 μM, lanes 3 and 6). Ethanol, the solvent for calpain inhibitor I and MG132, was added to the cells as control (lanes 1 and 4). Endogenous (A) and transfected (B and C) IRF-3 proteins were detected in whole cell extracts (20 μg) by immunoblot.

Figure 9. IRF-3 interacts with CBP in virus infected cells.

(A) Schematic representation of CBP, illustrating the domains involved in interaction with host or viral proteins (modified from (28)) and the myc-tagged CBP proteins (CBP1, CBP2, CBP3) used for immunoprecipitation.

(B) 293 cells were transfected with wild type and deletion mutants of IRF-3 expression plasmid (5 μg, as indicated above the lanes) or left untransfected (lanes 1 and 8). At 24h after transfection, cells were infected with Sendai virus for 16h (lanes 1, 3-8, and 10-13) or left uninfected (lanes 1 and 9). Whole cell extracts (300 μg, except lane 1, which was 600 μg) were immunoprecipitated with anti-CBP antibody A22 (lanes 1-6)

or with preimmune serum (lane 7). The immunoprecipitated complexes (lanes 1-7) or 30 μg whole cell extracts (lanes 8-13) were run on 5% SDS-PAGE and subsequently probed with anti-IRF-3 antibody.

(C) 293 cells were co-transfected with myc-tagged CBP expression plasmids (as indicated above the lanes) and IRF-3 ΔN (Δ9-133) expression plasmid. At 24h after transfection, cells were infected with Sendai virus (lanes 2, 4 and 6) or left uninfected (lanes 1, 3 and 5). Whole cell extracts (300 μg) were immunoprecipitated with monoclonal anti-myc-tag antibody 9E10. The immunoprecipitated complexes were run on 5% SDS-PAGE and different forms of IRF-3 in the precipitates were analyzed by immunoblotting with anti-IRF-3 antibody.

(D) Whole cell extracts (30 μg) from (C) were also analyzed directly for the expression of myc-tagged CBP proteins by immunoblotting using anti-myc antibody 9E10.

Figure 10. The cDNA sequence encoding IRF-3(5D), together with the amino acid sequence of IRF-3(5D).

Figure 11. Transactivation study as described in Figure 6, using the IFNβ-CAT reporter plasmid to indicate the activity of various forms of IRF-3 and IRF-7 and binary mixtures thereof.

Figure 12. The cDNA sequence encoding IRF-7A(2D), together with the amino acid sequence of IRF-7A(2D).

Figure 13. The cDNA sequence encoding the IRF-7(1-246)/IRF-3(5D)(132-427) chimeric protein, together with the amino acid sequence of the IRF-7(1-246)/IRF-3(5D)(132-427) chimeric protein.

Figure 14. Transactivation study as described in Figure 6, using the IFNβ-CAT reporter plasmid to indicate the relative activity of various forms of IRF-3 and IRF-7, binary mixtures thereof and the chimeric protein IRF-7(1-246)/IRF-3(132-427) (IRF-7N-IRF-3(5D)C in Figure 14).

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "nucleotide sequence" means a DNA or RNA molecule or sequence, and can include, for

example, a cDNA, genomic DNA, or synthetic DNA sequence, a structural gene or a fragment thereof, or an mRNA sequence, that encodes an active or functional polypeptide.

Two DNA, RNA or polypeptide sequences are "substantially homologous" or "structurally equivalent" when there is at least about 85% (preferably at least about 90%, more preferably at least about 95%) identity between the nucleotides or amino acids over a defined length of the molecule. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Appropriate hybridization conditions are within the knowledge of a person skilled in the art. See, for example, Maniatis et al., Molecular Cloning, A Laboratory Manual. Cold Spring Harbour Laboratory, New York (1982); Brown, T. A., Gene Cloning: An Introduction (2nd Ed.) Chapman & Hall, London (1990).

The results disclosed herein show that phosphorylation represents an important post-translational modification of IRF-3 leading to cytoplasmic-to-nuclear translocation of phosphorylated IRF-3, stimulation of DNA binding and transcriptional activity, association of IRF-3 with the transcriptional co-activator CBP/p300, and ultimately proteasome mediated degradation.

More specifically, the results disclosed herein show that, following Sendai virus infection, IRF-3 may be post-translationally modified by protein phosphorylation at multiple serine and threonine residues, located in the carboxy-terminus of IRF-3.

Furthermore, while modification of functionally relevant (phosphoacceptor) serine and threonine sites may be by phosphorylation, the modification may also be a mutation represented by replacement of at least one of these functionally relevant serine or threonine residues with an amino acid having a carboxylic acid in its side chain, preferably aspartic acid or glutamic acid, more preferably aspartic acid. The preferred mutant form of IRF-3 is that

having aspartic acid residues in at least one of postions 396, 398, 402, 404 and 405 of the sequence, more preferably in positions 396, 398, 402, 404 and 405 of the sequence (IRF-3(5D)) (Figure 10). The preferred mutant form of IRF-7 is that having aspartic acid residues in at least one of positions 477 and 479 of the sequence, more preferable in positions 477 and 479 of the sequence (IRF-7(2D)) (Figure 12).

Also within the scope of the invention are chimeric proteins comprising a carboxy-terminus domain of one modified IRF protein, modified as discussed above, and an amino-terminal domain of another IRF protein. Preferably, the amino-terminus of IRF-7 is fused to the carboxy-terminus of modified IRF-3. It is more preferred that the carboxy-terminus of modified IRF-3 is that of IRF-3(5D). Even more preferred is a chimeric protein comprising residues 1 to 246 of IRF-7 and residues 132 to 427 of IRF-3(5D) (Figure 13).

Also within the scope of the invention are proteins which are substantially homologous to the above proteins and which retain the function of those proteins.

Nucleotide sequences within the scope of the invention are those which encode a protein of the invention. Preferably, the nucleotide sequence is a coding DNA sequence as defined in Figure 10 or a DNA sequence which is hybridizable under stringent conditions with the complement of the coding DNA sequence of Figure 10, which DNA encodes IRF-3(5D). Also, preferably, the nucleotide sequence is a coding DNA sequence as defined in Figure 12 or a DNA sequence which is hybridizable under stringent conditions with the complement of the coding DNA sequence of Figure 12, which DNA encodes IRF-7(2D). Also preferably, the nucleotide sequence is a coding DNA sequence as defined in Figure 13 or a DNA sequence which is hybridizable under stringent conditions with the complement of the coding DNA sequence of Figure 13, which DNA encodes IRF-7(1-246)/IRF-3(132-427) chimeric protein.

A combination of IRF-3 deletion and point mutations localized the inducible phosphorylation sites to the region -ISNSHPLSLTSDQ- between amino acids 395 and 407; point mutation

of Ser-396 and Ser-398 residues eliminated virus-induced phosphorylation of IRF-3 protein, although residues Ser-402, Thr-404 and Ser-405 were also targets. Phosphorylation results in the cytoplasmic to nuclear translocation of IRF-3, DNA binding and increased transcriptional activation. Substitution of the Ser/Thr sites with the phosphomimetic Asp generated a constitutively active form of IRF-3 that functioned as a very strong activator of promoters containing PRDI/PRDIII or ISRE regulatory elements. Use of phosphomimetic Glu for this purpose is also possible. Phosphorylation also appears to represent a signal for virus mediated degradation, since the virus induced turnover of IRF-3 was prevented by mutation of the IRF-3 Ser/Thr cluster or by proteasome inhibitors.

Interestingly, virus infection resulted in the association of IRF-3 with the CBP coactivator, as detected by co-immunoprecipitation with anti-CBP antibody, an interaction mediated by the C-terminal domains of both proteins. Mutation of the residues Ser-396 and Ser-398 in IRF-3 abrogated its binding to CBP. These results are discussed in terms of a model in which virus-inducible C-terminal phosphorylation of IRF-3 alters protein conformation to permit nuclear translocation, association with transcriptional partners and primary activation of IFN- and IFN-responsive genes.

Sendai virus dependent phosphorylation of IRF-3 was detected, occurring in a cluster of Ser and Thr sites in the carboxyl-terminal end of the protein. The residues implicated in this regulatory phosphorylation event are Ser-396/Ser-398/Ser-402/Thr-404/Ser-405, particularly the Ser-396/Ser-398 amino acids. 2) Phosphorylation of the IRF-3 in the Ser-Thr cluster resulted in the cytoplasmic to nuclear translocation of IRF-3; nuclear translocation was blocked by mutation of the phosphorylated amino acids. 3) Sendai virus infection induced the DNA binding and transactivation potential of IRF-3. Furthermore, IRF-3 containing the phosphomimetic Asp at the sites of C-terminal phosphorylation was an exceptionally strong transactivator of PRDI/PRDIII and ISRE containing promoters. 4) Phosphorylation was also required for the

association of IRF-3 with the CBP co-activator protein. 5) Sendai virus infection resulted in IRF-3 degradation; again, phosphorylation was required as a signal for inducer mediated degradation since mutation of Ser/Thr cluster also blocked virus induced degradation.

Cytoplasmic to nuclear translocation of IRF-3 as a consequence of virus infection was inhibited by mutation of the Ser/Thr cluster, indicating an important regulatory role for C-terminal phosphorylation in the activation of IRF-3. Also strikingly, the conversion of the phosphorylation sites to the phosphomimetic Asp altered the subcellular localization of IRF-3 in uninfected cells. A proportion of IRF-3(5D) was localized to the nucleus of uninfected cells, suggesting that some IRF-3 may shuttle to and from the nucleus constitutively; this observation is consistent with the identification of a nuclear export signal in IRF-3. Mutation of L144A/L145A in the NES element produced the most impressive alterations in subcellular localization. In uninfected cells, IRF-3 was partitioned in both the nucleus and cytoplasm; virus infection changed the nuclear pattern of staining from extra-nucleolar homogeneous staining as observed for wtIRF-3 to an intense nuclear speckling. At this stage, the nature of the subnuclear changes in IRF-3 localization are not explained, although it is possible that IRF-3(NES) translocates efficiently into the nucleus but becomes trapped in the nuclear pore complex during the export process.

One of the striking results of the mutagenesis of the C-terminal domain of IRF-3 was the generation of IRF-3(5D), an exceptionally strong activator of IFN-β and ISG-15 gene expression. The phosphomimetic form of IRF-3 alone was able to stimulate IFN-β expression as strongly as virus infection, a level of stimulation not previously observed in co-expression experiments (24,61). In previous experiments, it has been demonstrated that IRF-3 was able to bind the ISRE element of ISG-15, as well as the PRDIII/PRDI and IE regions of the IFNB and IFNA promoters, respectively (2,56). Virus induction results in the appearance of two new protein-DNA complexes;

supershift experiments confirmed that both complexes contain IRF-3; it is not clear at this stage whether the upper complex also contains other proteins such as in the VIC (10,29) and DRAF (16) complexes or whether the lower complex represents a breakdown product of IRF-3. Strikingly, the same complexes appeared following co-transfection of IRF-3(5D) expression plasmid in the absence of virus induction, indicating that IRF-3(5D) represented a constitutive DNA binding form of IRF-3. Thus, in uninfected cells, IRF-3(5D) localized in part to the nucleus (Fig. 5), interacted with DNA constitutively and was a strong activator of gene expression (Fig. 6).

The recent crystal structure of the related IRF-1 protein bound to PRDI provides evidence for a novel helix-turn-helix motif that latches onto a GAAA core sequence via three of the five conserved tryptophan amino acids of the DNA binding domain (20). By analogy with IRF-3, two GAAANN sequences present in PRDIII of IFN-β and another GAAANN element present in PRDI may serve as DNA contacts for multiple IRF-3(5D) proteins with strong activating potential. Similarly, the ISRE element of the ISG-15 promoter also contains several GAAANN anchors for potential IRF binding. Given the range of promoters that possess this hexameric sequence (48), it will be of interest to determine the capacity of IRF-3(5D) to stimulate expression of different cytokine and chemokine genes.

IRF-3 joins a growing list of cellular and viral proteins that functionally interact with CBP/p300 proteins, highly homologous proteins originally identified through their interactions with adenovirus E1A and CREB proteins (1,13). As a critical determinant of its global transcriptional coactivator activity, CBP/p300 possesses histone acetyltransferase activity (5,50). Acetylation of histones is involved in the destabilization and remodelling of nucleosomes, a crucial step in permitting the accessibility of transcriptional factors to DNA templates. Several studies have now demonstrated that CBP/p300 participates in the transcriptional process by providing a scaffold for different

classes of transcriptional regulators on specific chromatin domains (12,50). A growing body of biochemical and genetic evidence also implicates CBP/p300 as a negative regulator of cell growth, based on its interactions with adenovirus E1a, SV40 large T antigen and the tumour suppressor p53, among others. With regard to p53-CBP/p300 complex formation, functional interaction between these two important growth regulatory proteins accounts for several of the known activities of p53 (3,28,40); interestingly, CBP/p300 was shown recently to acetylate p53 and stimulate its transactivation potential (27).

It will be of interest to determine whether IRF-3 is similarly modified by CBP association. The functional consequences of IRF-3 interaction with CBP/p300 remain to be elucidated, although recent studies demonstrated that CBP/p300 also functionally interacts with STAT 1 (68) and STAT 2 (7) and may contribute to IFNα and IFNγ nuclear signalling. Recently published studies have demonstrated that synergistic activation of the IFNβ promoter requires recruitment of CBP/p300 to the enhanceosome, via a new activating surface assembled from the activation domains of all the transcription factors in the enhanceosome (37,45). Alterations in any of the activation domains decreased both CBP recruitment and transcriptional synergy. By analogy, recruitment of CBP/p300 to DNA bound IRF-3 is likely required for maximal transcriptional activation. Association requires the interaction of the C-terminal domain of IRF-3 and the C-terminal interaction domain of CBP, a region previously shown to associate with the p53 tumour suppressor, whereas STAT1 and STAT2 associate with different regions of CBP (7,68).

Virus induced phosphorylation of IRF-3 also represents a signal for proteasome mediated degradation of IRF-3, since mutation of the Ser-396/Ser-398 or the use of proteasome inhibitors prevented the post infection degradation of IRF-3. Virus induced degradation of IRF-3 is reminiscent of the virus-induced turnover of another member of the IRF family - IRF-2. In response to dsRNA or viral induction, the 50 kD

IRF-2 protein is proteolytically processed into a smaller, 24-27 kDa protein (51) comprising the 160 aa DBD of IRF-2, termed TH3 (14) or In4 (65). Although TH3 has been shown to bind DNA and repress transcription more efficiently than the full length IRF-2 protein (42), its physiological role is not clear. Since the induction kinetics of TH3 are slower than that of IFN-β in response to dsRNA or viral infection (14), it has been suggested that the IRF-2 cleavage product may be a post-induction repressor of IFN-β gene expression (65).

Virus induced phosphorylation of IRF-3 at the C-terminal Ser/Thr residues and its subsequent degradation by a proteasome dependent pathway are also similar to the well studied phosphorylation and degradation of IκBα which leads to activation of NF-κB binding activity (reviewed in 4,6). In unstimulated cells, NF-κB heterodimers are retained in the cytoplasm by inhibitory IκB proteins. Upon stimulation by many activating agents, including cytokines, viruses and dsRNA, IκBα is rapidly phosphorylated and degraded, resulting in the release and nuclear translocation of NF-κB. The amino-terminus of IκBα represents a signal response domain for activation of NF-κB and substitution of alanine for either Ser-32 or Ser-36 completely abolished the signal-induced phosphorylation and degradation of IκBα, and blocked the activation of NF-κB. These mutations also blocked *in vitro* ubiquitination of the IκBα protein. The amino-terminus of IκBα is necessary for signal-induced phosphorylation and ubiquitination, but for degradation to occur, there is an absolute requirement for the C-terminal PEST domain (reviewed in 4,6).

Similarities and differences exist between the observed degradation of IRF-3 and the mechanism of IκBα degradation. The C-terminal phosphorylation of IRF-3 as a consequence of virus infection is required for its subsequent degradation based on the deletion and point mutation analysis of the region -ISNSHPLSLTSDQ- between amino acids 395 and 407. Minimally, phosphorylation of Ser-396 and Ser-398 are required for subsequent degradation, although Ser-402, Ser-404 and Ser-405 may represent secondary phosphorylation sites.

Likewise, in the case of IκBα, phosphorylation and Ser-32 and Ser-36 are required for inducer mediated degradation. Furthermore, the protease inhibitor calpain inhibitor I and the more specific proteasome inhibitor MG132 block IRF-3 turnover.

A major difference in the mechanisms of IκBα and IRF-3 turnover lies in the nature of the inducing stimuli. Multiple inducers - cytokines such as TNF and IL-1, viruses, LPS, oxidative stress, etc (6) - all lead to the induction of IκBα phosphorylation and degradation whereas IRF-3 phosphorylation appears to be induced only by virus infection and dsRNA addition; other inducers have not resulted in IRF-3 turnover.

A significant temporal difference also exists between IκBα phosphorylation/turnover and IRF-3 phosphorylation/degradation. Many activators of NF-κB stimulate IκBα phosphorylation within minutes and TNF induced degradation occurs within the first 15-30 minute after treatment. In the case of IRF-3, phosphorylation is not detected until 6-8 hours after infection and continues in a heterogenous manner over the next 10-12 hours. Previous experiments have, however, demonstrated that Sendai virus-induced turnover of IκBα also occurs slowly over several hours (24).

Based on the data presented herein and by analogy with the properties of other IRF family members (48), the following model is proposed to explain several observations. IRF-3 exists in a latent state in the cytoplasm of uninfected cells; the C-terminus may physically interact with the DNA binding domain in such a way as to obscure both the DBD and the IAD regions of the protein; the presence of an autoinhibitory domain within the C-terminal 20aa (407-427) would explain the activating effect of this deletion, as seen previously with IRF-4 (11,19). Virus induced phosphorylation at the Ser/Thr at 396-405aa cluster leads to a conformational change in IRF-3, exposing both the DBD and IAD and relieving C-terminal autoinhibition. Translocation to the nucleus, occurring via an

unidentified nuclear localization sequence or in conjunction with a transcriptional partner associating through the IAD region, leads to DNA binding at ISRE- and PRDI/PRDIII-containing promoters. Phosphorylation is also necessary for IRF-3 association with the chromatin remodelling activity of CBP/p300. The presence of a NES element ultimately shuttles IRF-3 from the nucleus and terminates the initial activation of IFN responsive promoters. The phosphorylated form of IRF-3 exported from the nucleus may now be susceptible to proteasome mediated degradation. This scenario shares several features with the protein synthesis independent activation of NF-κB, and further suggests that IRF-3 may represent a component of virus- or dsRNA-inducible complexes such as DRAF (16) or VIC (10,29) that could play a primary role in the induction of IFN- or IFN responsive genes.

In view of the above-mentioned properties, and in particular its ability to stimulate an immune response, IRF protein is useful as a tumour suppressor.

The invention is described in more detail in the following examples.

Example 1: Plasmid constructions and Mutagenesis.

The IRF-3 expression plasmid was prepared by cloning the *Eco*RI-*Xho*I fragment containing the IRF-3 cDNA from the pSKIRF-3 plasmid downstream of the CMV promoter of CMVBL vector. CMVt-IRF-3 was constructed by cloning of IRF-3 cDNA downstream of the doxycycline-responsive promoter CMVt at the *Bam*HI site of the neo CMVt BL vector (49). cDNAs encoding IRF-3 carboxyl terminal deletion mutations were generated by 28 cycles of PCR amplification with Vent DNA polymerase. DNA oligonucleotide primers were synthesized using an Applied Biosystems DNA/RNA synthesizer. The amino-terminal primer was synthesized with an *Eco*RI restriction enzyme site and the carboxyl-terminal primers were synthesized with *Xba*I restriction enzyme sites at their ends. The PCR products were purified by phenol/chloroform extraction and ethanol precipitation, digested with *Eco*RI and *Xba*I, and inserted into *Eco*RI/*Xba*I sites of CMVBL vector.

The point mutations of IRF-3 were generated by overlap PCR mutagenesis using Vent DNA polymerase. Mutations were confirmed by sequencing.

The N-terminal deletion mutations (ΔN, ΔN2A, ΔN3A and ΔN5A) of IRF-3 were generated by digestion of the related IRF-3/CMVBL plasmid with *Bam*HI (filled in with Klenow enzyme), partial digestion with *Sca*I, and re-ligation. GFP-IRF-3 expression plasmids were generated by cloning of cDNAs encoding wild type or mutated forms of IRF-3 into the downstream of EGFP in the pEGFP-C1 vector (Clonetech). For construction of plasmids encoding myc-tagged CBP truncated proteins, the cDNAs coding for CBP were generated from the pRC-RSV/mCBP plasmid (provided by Dr. Dimitris Thanos) by PCR amplification. The cDNA fragments were cloned in the downstream of myc-tag in 5′ myc-PCDNA3 vector (provided by Dr. Stephane Richard).

For the construction of pFlag-IRF-7, the IRF-7 cDNA was created by PCR and the resulting product was cloned into pFlag CMV-2 vector. To generate the IRF-7(aa1-246)-IRF-3(5D)(aa132-427) chimera, the cDNA encoding IRF-3 (5D) (aa132-427) was cut out from IRF-3 (5D)/CMVBL plasmid with ScaI and NotI (blunted with Klenow enzyme) and was cloned into pFlag-IRF-7 (digested with SmaI, which removed the C-terminal region of IRF-7 from 247-503) in frame with the IRF-7 N-terminal amino acid sequence (1-246). The point mutations of IRF-7 (D477-D479) were generated by overlap PCR mutagenesis essentially as described above for IRF-3 using Vent DNA polymerase. Codon AGC encoding residues Ser 477 and Ser 479 were mutated to GAC (Asp). Mutations were confirmed by sequencing.

Example 2: <u>Generation of IRF-3 cell lines.</u>

Plasmid CMVt-rtTA (49) was introduced into 293 cells by a calcium phosphate-based method. Cells were selected beginning at 48h after transfection for about one week in αMEM media (GIBCO-BRL) containing 10% heat-inactivated calf serum, glutamine, antibiotics and 2.5 ng/μl puromycin (Sigma). Resistant cells carrying the CMVt-rtTA plasmid (rtTA-293 cells) were then transfected with the CMVt-IRF-3 plasmid. Cells were selected beginning at 48h for a period of approximately 2 weeks

in αMEM containing 10% heat-inactivated calf serum, glutamine, antibiotics, 2.5 ng/μl puromycin and 400 μg/ml G418 (Life Technologies, Inc.).

Example 3: <u>Cell culture and transfections.</u>

All transfections for CAT assay were carried out in human embryonic kidney 293 cells or NIH3T3 cells grown in αMEM (293) or Dulbecco's MEM (NIH3T3) media (GIBCO-BRL) supplemented with 10% calf serum, glutamine and antibiotics. Subconfluent cells were transfected with 5 μg of CsCl purified chloramphenicol acetyltransferase (CAT) reporter and expression plasmids by calcium phosphate coprecipitation method (293 cells) or lipofectamine (NIH3T3 cells). The reporter plasmids were the SVoβ CAT and ISG15 CAT reporter genes (56); also the transfection procedures were previously described (41,56). For individual transfections, 100 μg (SVoβ CAT) or 10 μg (ISG15 CAT) of total protein extract was assayed for 1-2h at 37°C. The CAT activity was normalized with β-Gal assay. All transfections were performed 3-6 times.

Example 4: <u>Western blot analysis of IRF-3 modification and degradation.</u>

To characterize the posttranslational regulation of IRF-3 protein, stable or transiently transfected IRF-3 expressing cells were infected with Sendai Virus (80 HAU/ml) or treated with 5 ng/ml TNF-α, either with or without addition of 50 μg/ml cycloheximide. In some experiments, cells were treated with either 100 μM calpain inhibitor I (ICN), 40 μM MG132 proteasome inhibitor, or an equivalent volume of their respective solvent (ethanol) as control. Cells were washed with phosphate-buffered saline and lysed in 10 mm Tris-Cl pH 8.0, 200 mm NaCl, 1 mm EDTA, 1 mm dithiothreitol (DTT), 0.5% Nonidet P-40 (NP-40), 0.5 mm phenylmethysulfonyl fluoride (PMSF), 5 μg/ml leupeptin, 5 μg/ml pepstatin, and 5 μg/ml aprotinin. Equivalent amounts of whole cell extract (20 μg) were subject to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a 10% polyacrylamide gel. After electrophoresis, the proteins were transferred to Hybond transfer membrane (Amersham) in a buffer containing 30 mm This, 200 mm glycine

and 20% methanol for 1h. The membrane was blocked by incubation in phosphate-buffered saline (PBS) containing 5% dried milk for 1h and then probed with IRF-3 antibody in 5% milk/PBS, at a dilution of 1:3000. These incubations were done at 4°C overnight or at RT for 1-3h. After four 10 minute washes with PBS, membranes were reacted with a peroxidase-conjugated secondary goat anti-rabbit antibody (Amersham) at a dilution of 1:2500. The reaction was then visualized with the enhanced chemiluminescence detection system (ECL) as recommended by the manufacturer (Amersham Corp.).

Example 5: Phosphatase treatment.

Twenty to sixty μg of whole cell extract were treated with 0.3 units of potato acidic phosphatase (Sigma) in a final volume of 30 μl PIPES buffer (10 mm PIPES pH 6.0, 0.5 mm PMSF, 5 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin) or 5 units of calf intestine alkaline phosphatase (Pharmacia) in 30 μl CIP buffer. The phosphatase inhibitor mix contained 10 mm NaF, 1.5 mm Na_2MoO_4, 1 mm β-glycerophosphate, 0.4 mm Na_3VO_4 and 0.1 μg/ml okadaic acid.

Example 6: Subcellular localization of GFP-IRF-3 proteins.

To analyse the subcellular localization of wild type and mutated forms of IRF-3 proteins in uninfected and virus infected cells, the GFP-IRF-3 expression plasmids (5 μg) were transiently transfected into COS-7 cells by the calcium phosphate coprecipitation method. For virus infection, transfected cells were infected with Sendai virus (80 hemagglutinating units per mL for 2h) at 24h post transfection. GFP fluorescence was analyzed in living cells with a Leica fluorescence microscope using a 40x objective.

Example 7: Electromobility Shift Assay.

Nuclear extracts were prepared from 293 cells at different times after infection with Sendai virus (80HAU/ml). In some experiments, extracts were prepared from cells transfected with different IRF-3 expression plasmids, as indicated in individual experiments. Cells were washed in Buffer A [10 mM HEPES, pH 7.9; 1.5 mm $MgCl_2$; 10 mM KCl; 0.5 mM dithiothreitol (DTT); and 0.5 mM phenylmethylsulfonyl fluoride

(PMSF)] and were resuspended in Buffer A containing 0.1% NP-40. Cells were then chilled on ice for 10 minutes before centrifugation at 10,000 g. Pellets were then resuspended in Buffer B (20mM HEPES, pH 7.9; 25% glycerol; 0.42 M NaCl; 1.5 mM $MgCl_2$; 0.2 mM EDTA; 0.5 mM DTT; 0.5 mM PMSF; 5 μg/ml leupeptin; 5 μg/ml pepstatin; 0.5 mM spermidine; 0.15 mM spermine; and 5 μg/ml aprotinin). Samples were incubated on ice for 15 minutes before being centrifuged at 10,000 g. Nuclear extract supernatants were diluted with Buffer C (20 mM HEPES, pH 7.9; 20% glycerol; 0.2 mM EDTA; 50 mM KCl; 0.5 mM DTT; and 0.5 mM PMSF). Nuclear extracts were subjected to EMSA by using a 32P-labelled probe corresponding to the PRDIII region of the IFN-β promoter (5'-GGAAAACTGAAAGGG-3') or the ISRE region of the ISG-15 promoter (5'-GATCGGGAAAGGGAAACCGAAACTGAAGCC-3'). The resulting protein-DNA complexes were resolved by 5% polyacrylamide gel and exposed to X-ray film. To demonstrate the specificity of protein-DNA complex formation, 125-fold molar excess of unlabelled oligonucleotide was added to the nuclear extract before adding labelled probe.

Example 8: Immunoprecipitation and Western analysis of CBP associated proteins.

Whole cell extract (300 μg) were prepared from either transfected or untransfected cells and precleared with 5 μl of preimmune rabbit serum and 20 μl of protein A-Sepharose beads (Pharmacia) for 1 hour at 4°C. The extract was incubated with 10 μl of anti-CBP antibody A-22 (Santa Cruz) or 2 μl anti-myc antibody 9E10 (21) and 30 μl of protein A-Sepharose beads for 2-3 hours at 4°C. Precipitates were washed 5 times with lysis buffer, eluted by boiling the beads 3 minutes in 1x SDS sample buffer. Eluted proteins were separated by SDS PAGE, transferred to Hybond transfer membrane. Membranes were incubated with anti-IRF-3 (1:3000) or anti-myc antibody 9E10 (1:1000). Immunocomplexes were detected by using a chemiluminescence-based system.

The results of the above examples are summarized below.

Virus induced phosphorylation of IRF-3 protein.

IRF-3 is expressed constitutively in various cells and its expression is not enhanced by viral infection or by IFN treatment. To investigate whether the IRF-3 protein is regulated by post-translational modification after virus infection, 293 cells were transiently transfected with an IRF-3 expression plasmid and subsequently infected with Sendai virus 24h later. In cells transfected with CMVBL vector alone, endogenous IRF-3 protein was easily detected using a polyclonal IRF-3 antibody and in cells transfected with the IRF-3 expression plasmid, IRF-3 protein levels were significantly increased (Fig.1, lanes 1 and 3). Interestingly, Sendai virus infection resulted in two alterations in the expression of IRF-3: 1) an overall decrease in the amount of IRF-3 in transfected and control cells (Fig. 1, lanes 2 and 4) and the generation of a more slowly migrating form of IRF-3 (Fig. 1, compare lanes 1 and 2). In all experiments, the turnover of IRF-3 after virus infection was more pronounced with the endogenous protein than with the transfected proteins (see Fig.1, as well as others). Because the transfected proteins were driven by the CMV promoter, ongoing synthesis of transfected IRF-3 may partially obscure the turnover of IRF-3.

The kinetics of virus-induced modification of IRF-3 were characterized in a 293 cell line that expressed IRF-3 inducibly under the control of the tetracycline responsive promoter CMVt (25,26). Infection of this cell line (designated rtTA-IRF-3) with Sendai virus resulted in a decrease in the amount of IRF-3 between 12 and 24h after infection (Fig. 2A). Two forms of IRF-3 protein (designated I and II) were detected in uninfected cells (Fig. 2A, lane 1) and following virus infection, a third slowly migrating form of IRF-3 was also detected (Fig.2A, lanes 4-7). To determine whether the slowest form of IRF-3 was due to virus-induced phosphorylation (P-IRF-3), the different forms of IRF-3 were subjected to treatment *in vitro* with potato acidic phosphatase (PPA) or calf intestine alkaline phosphatase (CIP) and/or phosphatase inhibitors (Fig. 2B). These treatments did not affect the

mobilities of forms I and II in uninfected cells (Fig. 2B, lanes 1-3). However, in rtTA-IRF-3 expressing 293 cells infected with Sendai virus for 12h, an additional slowly migrating, presumably phosphorylated form of IRF-3 was also detected (Fig. 2B, lane 6); this form of IRF-3 completely disappeared following CIP or PPA treatment (Fig.2B, lanes 6 and 7) but was maintained in the presence of CIP/PPA when phosphatase inhibitors were also added to the reaction (Fig. 2B, lanes 5 and 8).

Mapping the IRF-3 phosphorylation sites.

A series of deletions of IRF-3 were generated to identify the virus-induced phosphorylation site(s) of IRF-3 (Fig. 3A). 293 cells were transiently transfected with IRF-3 deletion mutants and the virus mediated phosphorylation was measured by immunoblotting (Fig. 3B). The results indicated that a virus-induced phosphorylation of IRF-3 occurs at the C-terminal end of IRF-3 since the mutations that contained only the N-terminal part of IRF-3 protein (133, 240, 328, 357 or 394aa) were not phosphorylated (Fig. 3B). Full length and 407aa forms of IRF-3 were phosphorylated as a consequence of virus infection (Fig. 3B, lanes 1-4). C-terminal truncation of IRF-3 to a protein of 394 or 357aa removed the site(s) of inducible phosphorylation (Fig. 3B, lanes 5-8), although the shortened versions of forms I and II were still observed. Also in the IRF-3 Δ9-133 mutation (ΔN) which had the DNA binding, N-terminal amino acids (aa9 to aa133) removed, both virus induced phosphorylation of IRF-3 and the differential migration of the shortened forms I and II were easily detected (Fig. 3B, lanes 9 and 10). Degradation of the endogenous forms of IRF-3 by virus infection was also detected in this experiment (compare Fig. 3B, lanes 7 and 9 with lanes 8 and 10).

Thus, by deletion analysis, a phosphorylation domain of IRF-3 protein was localized to the region -ISNSHPLSLTSDQ- between amino acids 395 and 407. Point mutations in the several putative Ser and Thr phosphorylation residues within this region were generated in the full length protein and the Δ9-133 (ΔN) protein (Fig. 4A). In the IRF-3 cDNA encoding

these proteins, the Ser-396/Ser398/Ser-402/Thr-404/Ser-405 residues were replaced by alanine (5A), as were the three residues Ser-402/Thr-404/Ser-405 (3A) and the two residues Ser-396/Ser-398 (2A). Transfection of these plasmids into 293 cells and subsequent virus infection revealed that full length wild type IRF-3 was phosphorylated (Fig. 4B, lanes 4 and 8), whereas the IRF-3 proteins containing 2A and 5A mutations were no longer phosphorylated in virus infected cells (Fig. 4B, lanes 6 and 10). Interestingly, IRF-3-3A was also very weakly phosphorylated as a consequence of virus infection, thus implicating Ser-402/Thr-404/Ser-405 as potential secondary sites of phosphorylation. Using the ΔN IRF-3 protein and the relevant point mutations, phosphorylation was detected with ΔN (Fig. 4B, lane 12) but not with ΔN-2A and ΔN-5A (Fig. 4B, lanes 14 and 18); likewise, ΔN-3A displayed very weak phosphorylation (Fig. 4B, lane 16).

These experiments thus implicate Ser-396 and Ser-398 as critical sites of virus-induced phosphorylation of IRF-3; however, Ser-402/Thr-404/Ser-405 residues also contribute to the observed phosphorylation, since the migration of phosphorylated ΔN-3A is significantly faster than ΔN and the phosphorylation level is decreased (Fig. 4B, lanes 12 and 16). Another study suggested the involvement of the Ser residues at aa385 and 386 as potential phosphoacceptor sites (67). However, in studies with the S385A/S386A mutation, no evidence was found for inducible phosphorylation at these sites. Nevertheless, since these sites represent consensus sites for CKI and CKII, constitutive phosphorylation is a possibility.

<u>IRF-3 phosphorylation induces cytoplasmic to nuclear translocation of IRF-3.</u>

Initial studies indicated that IRF-3 was localized in the cytoplasm of uninfected cells (67); to investigate the role of phosphorylation on IRF-3 localization, wild type and point mutated forms of IRF-3 were linked to green fluorescent protein (GFP), transfected into COS-7 cells and examined for Sendai virus induced changes in subcellular localization (Fig. 5). In uninfected cells, GFP-IRF-3 localized exclusively to the

cytoplasm; Sendai virus infection resulted in translocation of IRF-3 to the nucleus within 8h in 90-95% of the cells (Fig. 5A and B). Mutation of the Ser/Thr cluster in GFP-IRF-3(5A) completely abrogated virus-induced cytoplasmic to nuclear translocation (Fig. 5, C and D). Interestingly, the substitution of the Ser/Thr cluster with the phosphomimetic Asp in GFP-IRF-3(5D) likewise altered subcellular localization. IRF-3(5D) localized both to the nucleus and cytoplasm in uninfected cells (Fig. 5E), while virus infection resulted in an intense nuclear pattern of IRF-3(5D) fluorescence (Fig. 5F). Point mutation of a putative nuclear export signal in IRF-3, the L145A/L146A modification - termed IRF-3(NES) - also changed subcellular localization of IRF-3. In uninfected cells, GFP-IRF-3(NES) was localized to the nucleus and cytoplasm, with a homogeneous, extra-nucleolar pattern of nuclear staining. After virus infection, GFP-IRF-3(NES) localized to the nucleus with an intense speckled pattern of nuclear fluorescence in greater than 95% of the cells, suggesting that IRF-3(NES) may be trapped in the nucleus associated with the nuclear pore complex.

<u>Transactivation of PRDI/PRDIII and ISRE promoters by IRF-3.</u>

Next, the capacity of IRF-3 to regulate gene expression was analysed by transient transfection in human 293 and murine NIH3T3 cells using the IFNβ and ISG-15 promoters in reporter gene assays. Expression of NF-κB RelA(p65), IRF-1 and IRF-3 alone minimally induced IFNβ promoter activity between 3 to 4 fold (Fig. 6A and B), as shown previously (24,56,61). Introduction of the C-terminal point mutants - IRF-3(2A), IRF-3(3A) IRF-3(5A) - reduced the low transactivation capacity of IRF-3 to control levels (Fig. 6A). Interestingly, deletion of the C-terminal 20aa of IRF-3 to IRF-3(407) stimulated IFNβ activity about 6 fold, indicative of the removal of an inhibitory domain in IRF-3. However, further deletion to 394, 357 or 240 abrogated transactivation potential (Fig. 6A). Mutation of the NES element was not sufficient to stimulate IFNβ activity. Strikingly, the substitution of the Ser/Thr cluster at aa397-405 in IRF-3 with the phosphomimetic Asp

generated a very strong, constitutive transactivator protein that alone stimulated the IFNβ promoter 90 fold.

As shown previously, high level induction of the IFNβ promoter requires synergistic activation by NF-κB and IRF proteins (24,61). To analyse the properties of IRF-3 in synergistic activation of the IFNβ promoter, co-expression studies were performed using RelA(p65) expression plasmid and different wild type and mutant forms of IRF-3 (Fig. 6B). Co-expression of RelA and IRF-1 or RelA and IRF-3 stimulated IFNβ-CAT activity by 20-25 fold. IRF-3(407) and RelA(p65) stimulated IFNβ activity about 40 fold, supporting the idea of the removal of an inhibitory domain in IRF-3, whereas both the IRF-3(394) and the IRF-3(NES) failed to synergise with RelA in the activation of the IFNβ promoter. RelA and IRF-3(NES) produced a relatively weak 8 fold induction of IFNβ expression, indicating that nuclear localization is not sufficient for IRF-3 activation. The combination of RelA and IRF-3(5D) produced an 80 fold stimulation of IFNβ promoter activity (Fig. 6B); together with the above data, IRF-3(5D) alone appears to be capable of full stimulation of the IFNβ promoter and further synergy with RelA is not observed (compare Fig. 6A and B). Surprisingly, IRF-3(5A) and RelA produced a 30 fold stimulation, suggesting that 5A can still synergise with RelA, despite mutation of the Ser/Thr cluster.

The transactivation potential of IRF-3 was also analysed using the ISG-15 promoter, an ISRE containing regulatory element (Fig. 6C). As shown previously (2), and above for the IFNβ promoter, IRF-3 alone weakly activated the ISG-15 promoter; in the context of this regulatory element, IRF-3 was weaker than IRF-1, which produced a 9 fold stimulation. Again deletion of the C-terminal 20aa of IRF-3 generated a protein that stimulated gene expression; with the ISG-15 promoter, a 12 fold induction was observed; IRF-3(394) and IRF-3(357) did not stimulate gene expression but rather slightly repressed ISG-15. Again remarkably, IRF-3(5D) produced a 50 fold induction of the ISG-15 promoter (Fig. 6C), thus demonstrating that substitution of the Ser/Thr sites with

the phosphomimetic Asp generated a constitutively active form of IRF-3 that functioned as a very strong activator of promoters containing PRDI/PRDIII or ISRE regulatory elements.

Activation of RANTES Transcription by IRF-3 and Virus

Chemokine expression is demonstrated in Figure 7, the chemokine being RANTES (Regulated on Activation Normal T-cell Expressed and Secreted) protein. IRF-3-inducible cells were used to determine whether other cytokine-chemokine genes may be regulated by IRF-3; an (Rnase Protection Analysis (RPA) with multiple human cytokine-chemokine probes (Pharmingen) was used to examine RNA derived from rtTA-IRF-3 or rtTA-IRF-3(5D) cells. Strikingly, the RANTES gene was highly expressed in the IRF-3(5D)-inducible cells, as well as in virus-infected cells (Fig. 7A, lanes 3, 5, and 7) but not in uninfected rtTA- or wt IRF-3-expressing cells (Fig. 7A, lanes 1 and 4). Since IRF-3(5D) was a strong transactivator of the IFN-β promoter in transient transfection assays, the possibility of an autoregulatory effect of IFN-α/β expression on transcription of RANTES promoter via JAK-STAT activation was considered. Activation of RANTES did not occur secondary to the production of IFN-α/β, since RANTES mRNA was not detected in control rtTA-expressing cells treated directly with IFN-α/β (Fig. 7A, lane 2); furthermore, addition of neuralizing antibody directed against type I IFN did not block the stimulation of RANTES gene expression by IRF-3(5D) (Fig. 7A, lane 8). Other experiments also demonstraed that IRF-3 itself was not activated by IFN treatment (13a). Inducible expression of RANTES in cells stably expressing a dominant-negative form of IRF-3 which lacks the N-terminal amino acids 9 to 133 and does not bind to DNA was also examined. As shown in Fig. 7B, RANTES gene transcription was indcued by Sendai virus in control (rtTA) cells (Fig. 7B) but not in IRF-3 (ΔN)-expressing cells (Fig. 7B). This experiment indicates that a non-DNA binding, dominant-negative mutant of IRF-3 is able to block completely virus-induced activation of RANTES transcription.

The kinetics of IRF-3 transgene induction and RANTES mRNA expression were characterized at various times following

Dox induction. IRF-3(5D) was detected at 8 to 12 hours with peak levels at 24 hours following Dox addition. RANTES mRNA was first detectable at 18 hours after Dox induction with peak levels at 40 hours (Fig. 7C, lanes 5 to 10). Induction of RANTES protein expression as detected by ELISA (Fig. 7D) was first observed at 12 hours after Dox induction of IRF-3, in good agreement with the mRNA levels, and accumulated thereafter with a dramatic increase between 24 and 32 hours after stimulation, also in agreement with mRNA levels. The possibility that IRF-3(5D) may be directly activating another transcription factor such as NF-κB, which in turn would stimulte RANTES transciption, was also considered. No evidence for IRF-3(5D)-mediated activation of NF-κB DNA binding activigy was observed. Similarly, IRF-3(5D) expression did not activate the human immunodeficiency virus (HIV)-long terminal repeat, a complex promoter controlled by NF-κB and other transcription factors (data not shown).

Inhibition of IRF-3 degradation.

Another consequence of virus infection is the degradation of the IRF-3. Since phosphorylation of proteins is functionally associated with the process of protein degradation via the ubiquitin-dependent proteasome pathway (53,57,60), the effect of proteasome inhibitors on virus-induced turnover of IRF-3 was examined. In cells transfected with the ΔN and ΔN5A forms of IRF-3, virus induced degradation of full length (endogenous) forms of IRF-3 (Fig. 8A, lanes 1 and 4) and the truncated ΔN (Fig. 8B, lanes 1 and 4) was detected. Addition of the protease inhibitor calpain inhibitor I or the proteasome inhibitor MG132 blocked virus-induced IRF-3 degradation (Fig. 8A and 8B, lanes 4-6). Particularly with the ΔN protein, the accumulation of the phosphorylated form of ΔN was also detected in virus infected cells (Fig. 8B, lanes 5 and 6), suggesting that phosphorylation of IRF-3 may represent a signal for subsequent degradation by the proteasome pathway. To confirm this idea, the 5A point mutated form of IRF-3 was analysed; the IRF-3-ΔN5A protein was resistant to virus induced degradation (Fig. 8C, lanes 1 and 4); no further stabilization of

IRF-3-ΔN5A occurred with calpain inhibitor I or MG132 addition and no phosphorylated IRF-3 was detected (Fig. 8C, lanes 4-6). These experiments demonstrate that virus dependent phosphorylation of the C-terminal of IRF-3 represents a signal for subsequent proteasome mediated degradation.

Interaction between IRF-3 and CBP in virus infected cells.

To examine the possibility that IRF-3 associated with the co-activator CBP/p300 (Fig. 9A) following Sendai virus infection, CBP was immunoprecipitated from virus-infected cells with anti-CBP antibody; an immunoblot for IRF-3 revealed that IRF-3 was co-precipitated from virus-infected cells but not from uninfected cells (Fig. 9B, lanes 2 and 3). This interaction was observed clearly in cells co-transfected with the IRF-3 expression plasmid (Fig. 9B, lane 3) but was not seen when the immunoprecipitation was performed with pre-immune serum (Fig. 9B, lane 7). The endogenous IRF-3 also co-precipitated from virus-infected cells (Fig. 9B, lane 1). However, mutation of the Ser/Thr residues identified as the virus inducible phosphorylation sites abrogated the association of IRF-3 with CBP. In particular, IRF-3(2A) and IRF-3(5A) were detected in whole cell extract immunoblot but not in the CBP immunoprecipitate (Fig. 9B, compare lanes 4 and 6 with lanes 11 and 13). With the IRF-3(3A) mutant, interaction with CBP was still observed (Fig. 9B, lane 5). The high background in all lanes represents secondary antibody reactivity with rabbit IgG from the immunoprecipitation. Immunoblot analysis of the whole cell extracts revealed that phosphorylated IRF-3, as well as forms I and II were present in virus infected cells (Fig. 9B, lane 10) and in cells transfected with 2A, 3A and 5A the forms I and II were observed but not the phosphorylated form of IRF-3 (Fig. 9B, lanes 11-13).

CBP has several domains that bind transcription factors, designated CBP1, CBP2, and CBP3 respectively (Fig. 9A, reviewed in (28)). To determine which domain of CBP interacts with IRF-3, the three specific subdomains were myc-tagged at the 5′ end by subcloning into the pCDNA3 vector (Fig. 9A). 293 cells were co-transfected with these myc-tagged CBP expression

plasmids together with the IRF-3 ΔN (Δ9-133) expression plasmid. At 24h after transfection, cells were infected with Sendai virus, co-immunopreciptated with anti-myc antibody 16h later (21) and then immunoblotted for IRF-3. Endogenous IRF-3 and transfected IRF-3 ΔN proteins co-precipitated with CBP-3 from virus-infected cells but not from uninfected cells (Fig. 9C, lane 6). In cells co-transfected with CBP-1 and CBP-2, no endogenous or transfected ΔN IRF-3 was detected (Fig. 9C, lanes 1-4). Immunoblot analysis of the whole cell extracts revealed that all three myc-tagged CBP proteins were efficiently expressed in uninfected and virus infected cells (Fig. 9D). These results demonstrate that IRF-3 binds to the C-terminal domain of CBP in virus infected cells and interaction with CBP requires Ser-396/Ser-398 phosphorylation of IRF-3 but not at Ser-402/Thr-404/Ser-405.

Figure 11 shows the relative activity of various forms of IRF-3 and IRF-7, and binary mixtures thereof, in transactivation studies. Both the IRF-3(5D) and IRF-7(2D) mutants show increased activity relative to their corresponding wild-type proteins. There is a synegistic effect present when both proteins are present, and this effect is most pronounced in a mixture of the IRF-3(5D) and IRF-7(2D) (D477/479) mutants.

Figure 14 shows that the chimeric protein IRF-7(1-246)/IRF-3(5D)(132-427) has a markedly increased activity over the mixture of the IRF-3(5D) and IRF-7(2D) (D477/479) mutants.

A pharmaceutical composition may be prepared, with a protein of the invention as active ingredient, for the treatment of a viral infection, such as an influenza infection, a herpes infection or an HIV infection.

The pharmaceutical compositions of the present invention may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers. Thus, the active compounds of the invention may be formulated for oral, buccal, transdermal (e.g., patch), intranasal, parenteral (e.g., intravenous, intramuscular or subcutaneous) or rectal

administration or in a form suitable for administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycollate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); and preservatives (e.g., methyl or propyl p-hydroxybenzoates or sorbic acid).

For buccal administration the composition may take the form of tablets or lozenges formulated in conventional manner.

The active compounds of the invention may be formulated for parenteral administration by injection, including using conventional catherization techniques or infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for

reconstitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The active compounds of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

For intranasal administration or administration by inhalation, the active compounds of the invention are conveniently delivered in the form of a solution or suspension from a pump spray container that is squeezed or pumped by the patient or as an aerosol spray presentation from a pressurized container or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

The protein of the invention can also be made available using gene therapy. The DNA encoding the protein can be introduced to cells of an organism at a target site, for example, by a viral vector, by electroporation, by co-transfection with a selectable marker, or by DNA vaccine.

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Claims:

1. A modified interferon regulatory factor (IRF) protein, the protein comprising at least one modified serine or threonine phosphoacceptor site in the carboxy-terminus domain.

2. The interferon regulatory factor (IRF) protein according to claim 1, wherein cytokine gene activation by the modified IRF is increased relative to cytokine gene activation by a corresponding wild type IRF protein.

3. The interferon regulatory factor (IRF) protein according to claim 1 or 2, wherein the at least one modified phosphoacceptor site is modified by phosphorylation.

4. The interferon regulatory factor (IRF) protein according to claim 1 or 2, wherein the at least one modified phosphoacceptor site comprises an amino acid residue having an acidic side chain.

5. The interferon regulatory factor (IRF) protein according to claim 4, wherein the amino acid residue is aspartic acid.

6. The interferon regulatory factor (IRF) protein according to claim 3, 4 or 5, wherein the modified IRF is IRF-3 modified at a site selected from at least one of Ser-396, Ser-398, Ser-402, Thr-404 and Ser-405.

7. The interferon regulatory factor (IRF) protein according to claim 6, wherein the modified IRF is IRF-3 modified at Ser-396, Ser-398, Ser-402, Thr-404 and Ser-405 sites.

8. The interferon regulatory factor (IRF) protein according to claim 7 having the sequence of ID No. 2 in the sequence listing (IRF-3(5D)).

9. The interferon regulatory factor (IRF) protein according to claim 7, wherein the modified IRF comprises a carboxy-terminus domain of IRF-3 modified at a site selected from at least one of Ser-396, Ser-398, Ser-402, Thr-404 and Ser-405 and an amino-terminus domain from IRF-7.

10. The interferon regulatory factor (IRF) protein according to claim 9, wherein the modified IRF has an amino-terminal domain comprising residues 1 to 246 of IRF-7 and a carboxy-terminal domain comprising residues 132 to 427 of IRF-3 modified by replacement of each of Ser-396, Ser-398, Ser-402, Thr-404 and Ser-405 by an aspartic acid residue.

11. The interferon regulatory factor (IRF) protein according to claim 10 having the sequence of ID No. 11 in the sequence listing (IRF-7(1-246)/IRF-3(5D)(132-427)).

12. The interferon regulatory factor (IRF) protein according to claim 3, 4 or 5, wherein the modified IRF is IRF-7 modified at a site selected from at least one of Ser-477 and Ser-479.

13. The interferon regulatory factor (IRF) protein according to claim 12, wherein the modified IRF-7 is modified at Ser-477 and Ser-479 sites.

14. The interferon regulatory factor (IRF) protein according to claim 13 having the sequence of ID No. 9 in the sequence listing (IRF-7(2D)).

15. A nucleotide sequence which encodes the interferon regulatory factor (IRF) protein according to any one of claims 1 to 14, or a nucleotide sequence that is hybridizable under stringent conditions with the complement of the nucleotide sequence which encodes the interferon regulatory factor (IRF) protein.

16. The nucleotide sequence according to claim 15, which is a DNA sequence of ID No. 1 in the sequence listing.

17. The nucleotide sequence according to claim 15, which is a DNA sequence of ID No. 8 in the sequence listing.

18. The nucleotide sequence according to claim 15, which is a DNA sequence of ID No. 10 in the sequence listing.

19. A pharmaceutical composition comprising an effective amount of the interferon regulatory factor (IRF) protein according to any one of claims 1 to 14, together with a pharmaceutically acceptable carrier, for the treatment of a viral infection.

20. The pharmaceutical composition according to claim 19, wherein the viral infection is selected from an influenza infection, a herpes infection and an HIV infection.

21. Use of the interferon regulatory factor (IRF) protein according to any one of claims 1 to 14 to activate a cytokine gene.

22. The use according to claim 21, wherein the cytokine gene is an interferon gene or a chemokine gene.

23. Use of the interferon regulatory factor (IRF) protein according to any one of claims 1 to 14 in cancer treatment.

24. Use of the nucleotide sequence according to any one of claims 15 to 18 to modify a target cell of an organism.

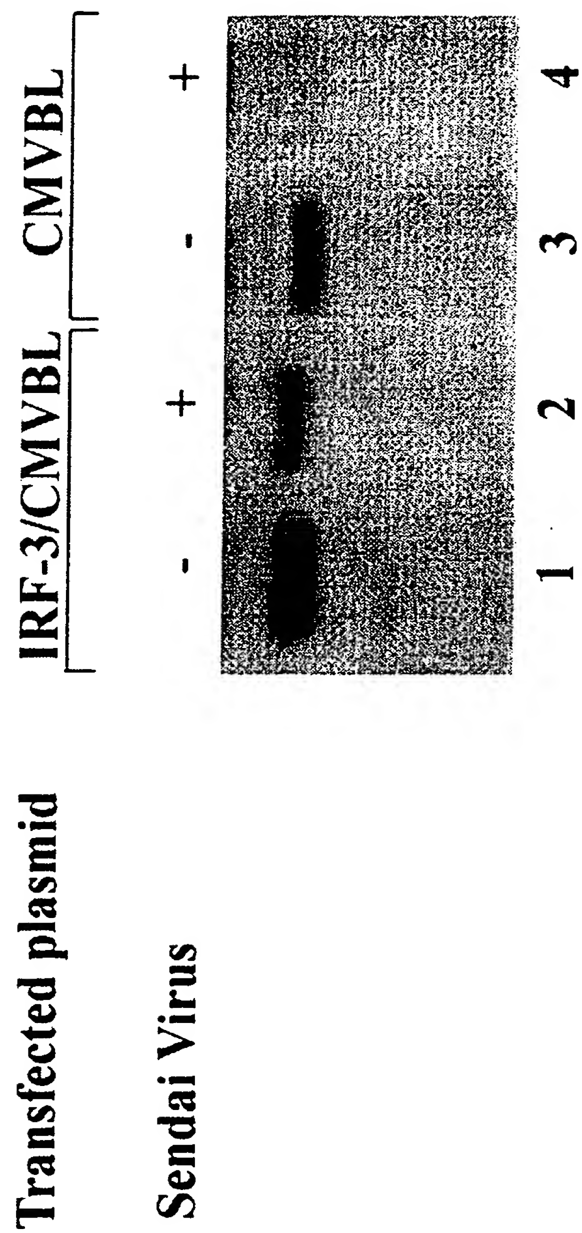

FIG. 1

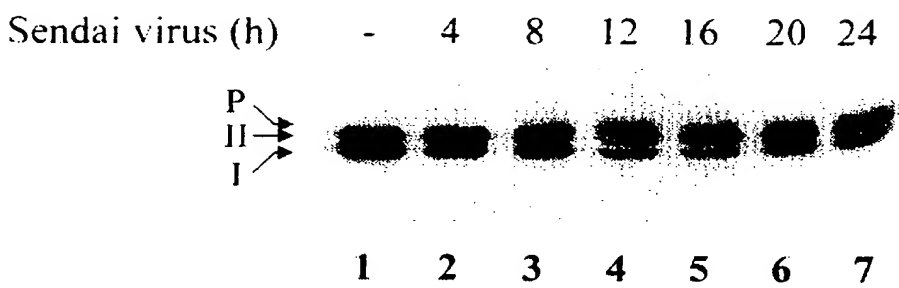
Sendai virus (h) - 4 8 12 16 20 24
P
II
I
1 2 3 4 5 6 7

FIG. 2A

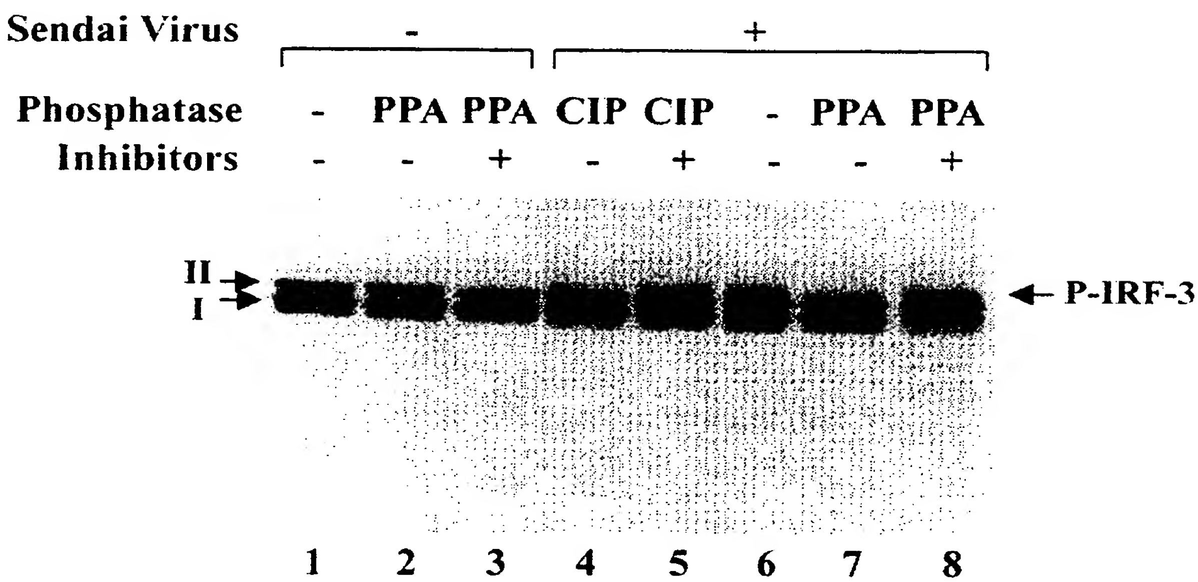
Sendai Virus - +
Phosphatase - PPA PPA CIP CIP - PPA PPA
Inhibitors - - + - + - - +
II
I
P-IRF-3
1 2 3 4 5 6 7 8

FIG. 2B

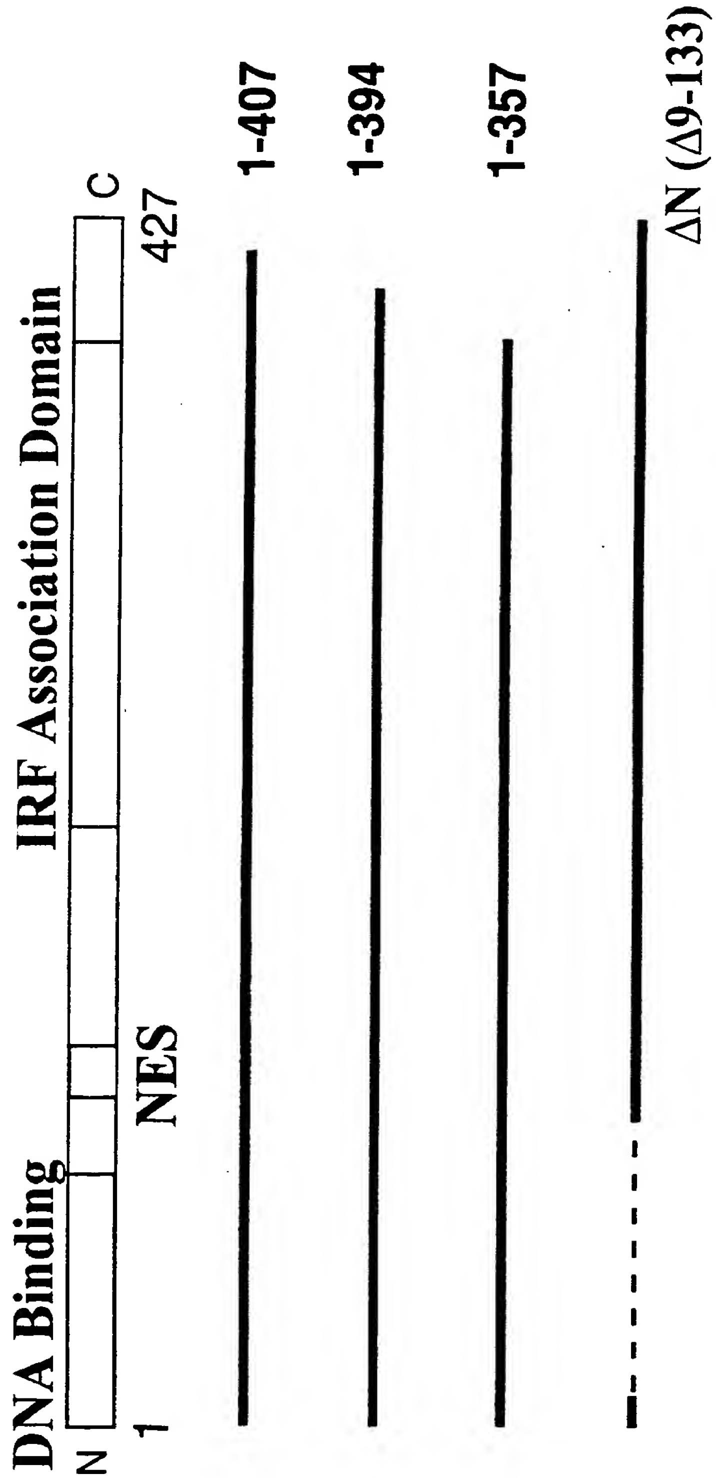
DNA Binding
NES
IRF Association Domain
N
C
1
427
1-407
1-394
1-357
ΔN (Δ9-133)

FIG. 3A

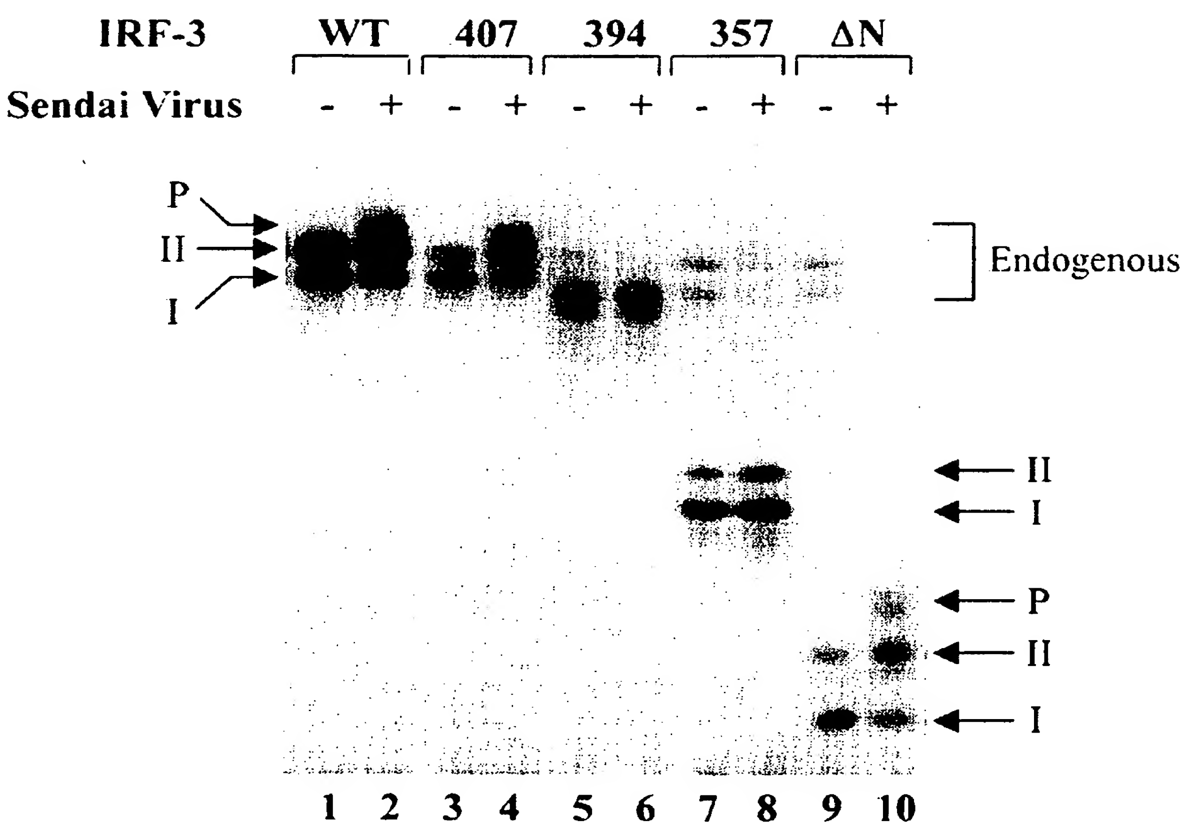
IRF-3
WT
407
394
357
ΔN
Sendai Virus
- + - + - + - + - +
P
II
I
Endogenous
II
I
P
II
I
1 2 3 4 5 6 7 8 9 10

FIG. 3B

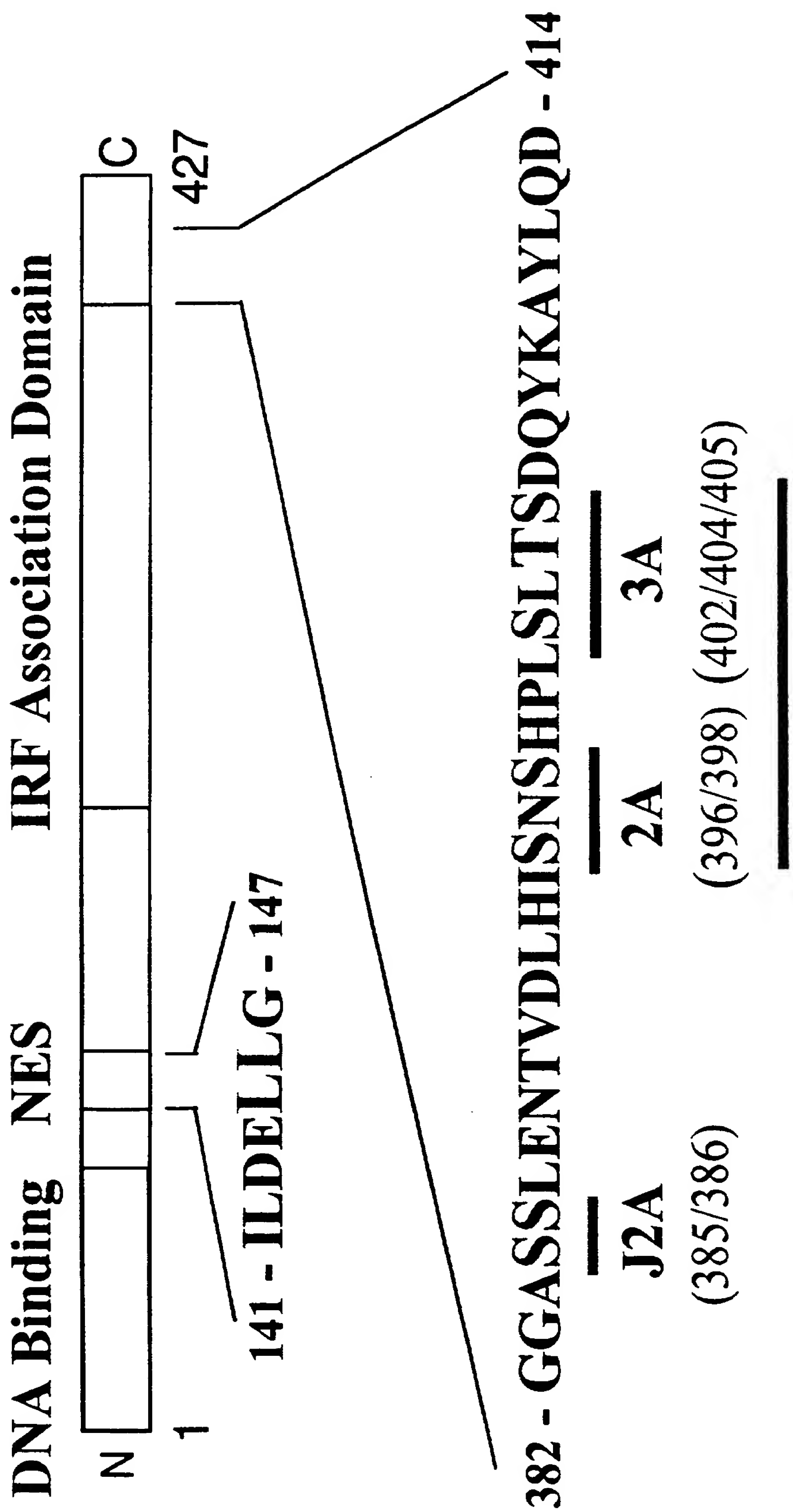
DNA Binding
NES
IRF Association Domain
N
C
1
427
141 - ILDELLG - 147
382 - GGASSLENTVDLHISNSHPLSLTSDQYKAYLQD - 414
J2A
(385/386)
2A
(396/398)
3A
(402/404/405)
5A
5D

FIG. 4A

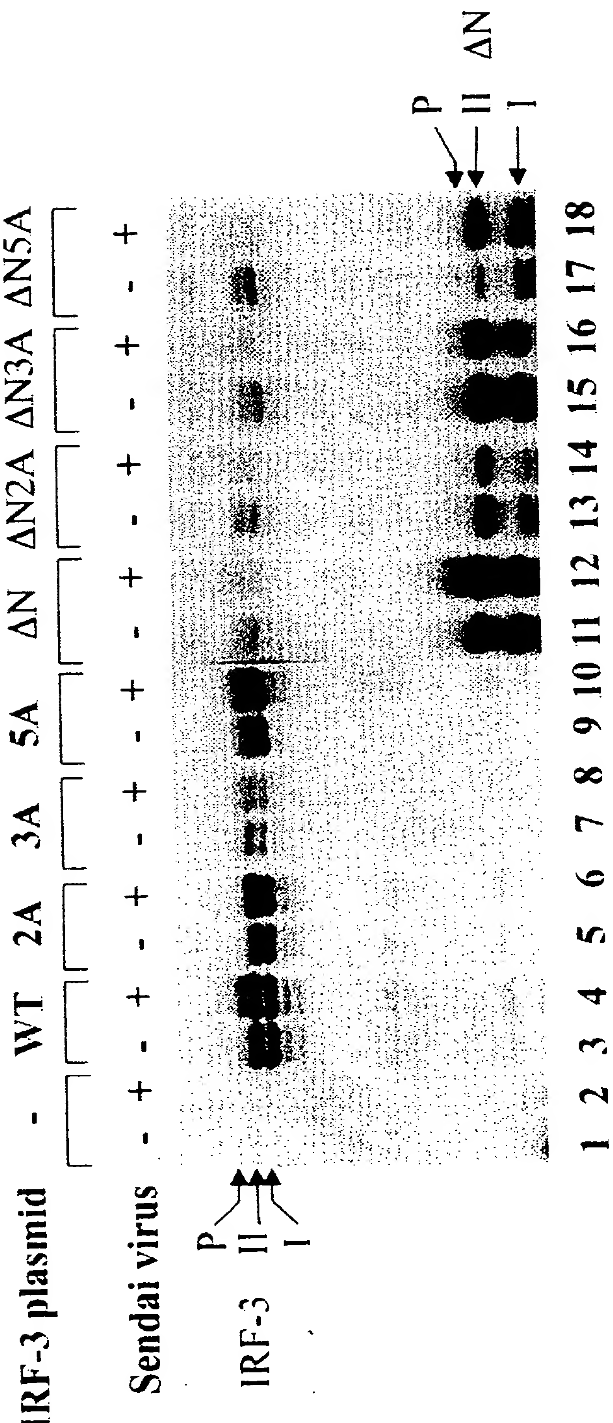
IRF-3 plasmid
- WT 2A 3A 5A ΔN ΔN2A ΔN3A ΔN5A
Sendai virus
- + - + - + - + - + - + - + - + - +
IRF-3
P
II
I
P
II
I
ΔN
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

FIG. 4B

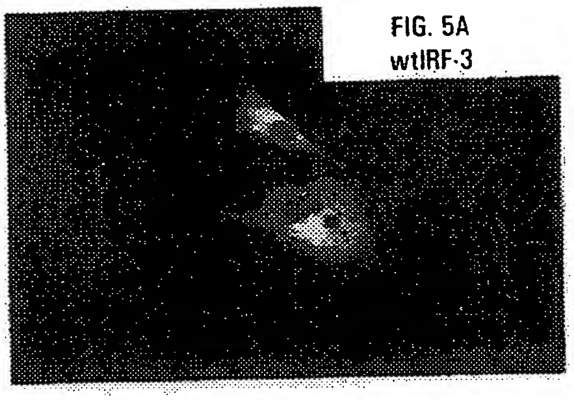

FIG. 5A
wtIRF-3

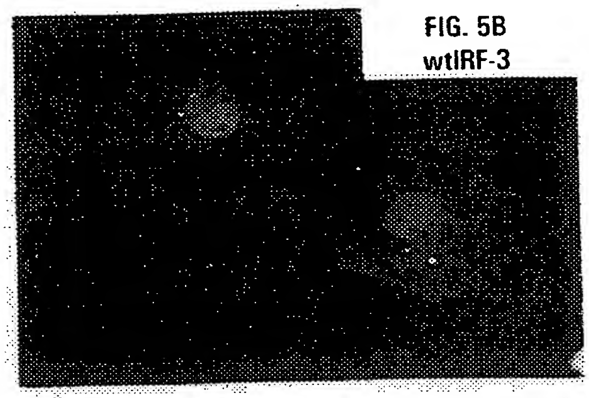

FIG. 5B
wtIRF-3

FIG. 5C
IRF-3(5A)

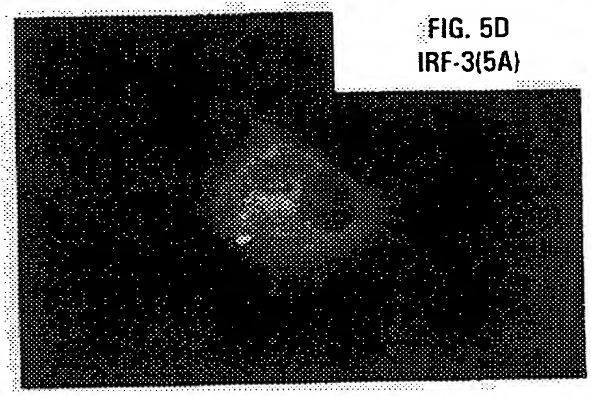

FIG. 5D
IRF-3(5A)

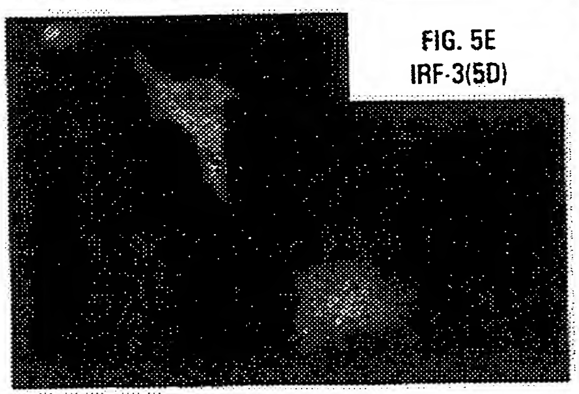

FIG. 5E
IRF-3(5D)

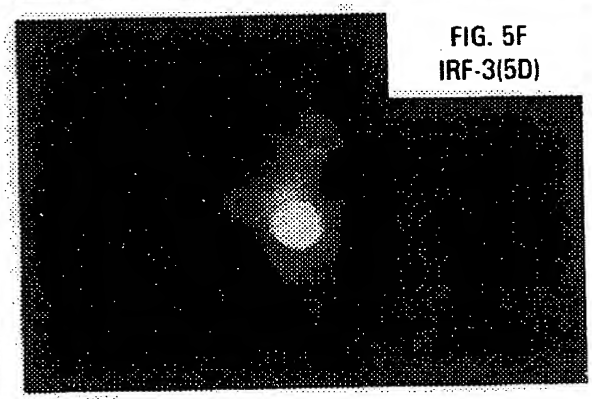

FIG. 5F
IRF-3(5D)

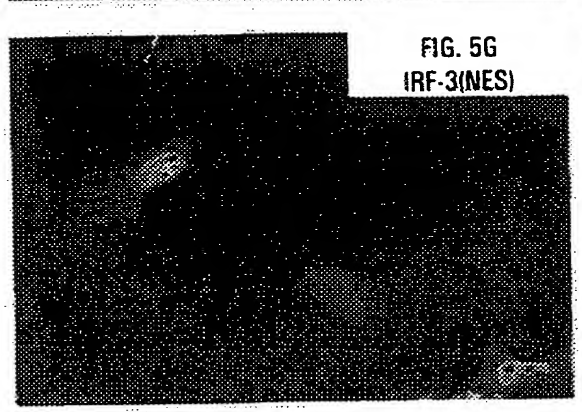

FIG. 5G
IRF-3(NES)

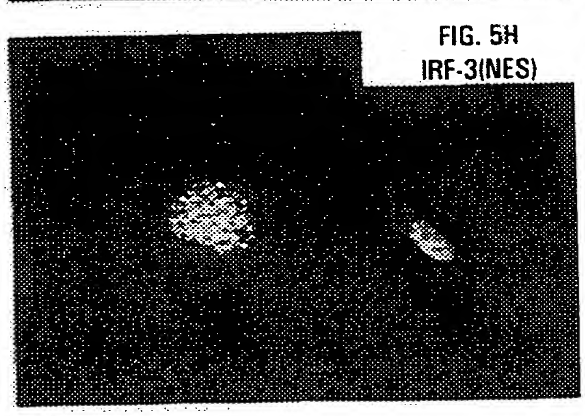

FIG. 5H
IRF-3(NES)

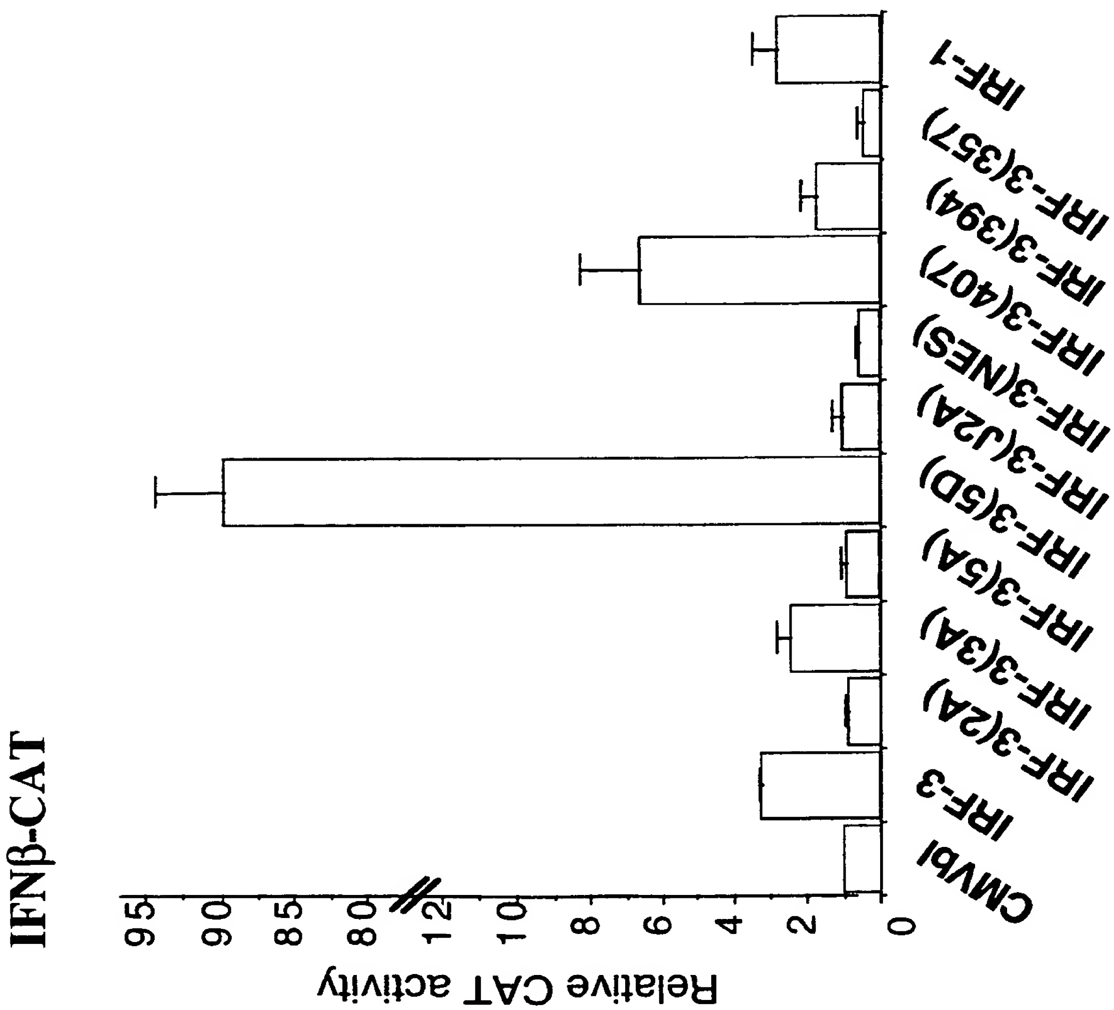
IFNβ-CAT
Relative CAT activity
0
2
4
6
8
10
12
80
85
90
95
CMVbl
IRF-3
IRF-3(2A)
IRF-3(3A)
IRF-3(5A)
IRF-3(5D)
IRF-3(J2A)
IRF-3(NES)
IRF-3(407)
IRF-3(394)
IRF-3(357)
IRF-1

FIG. 6A

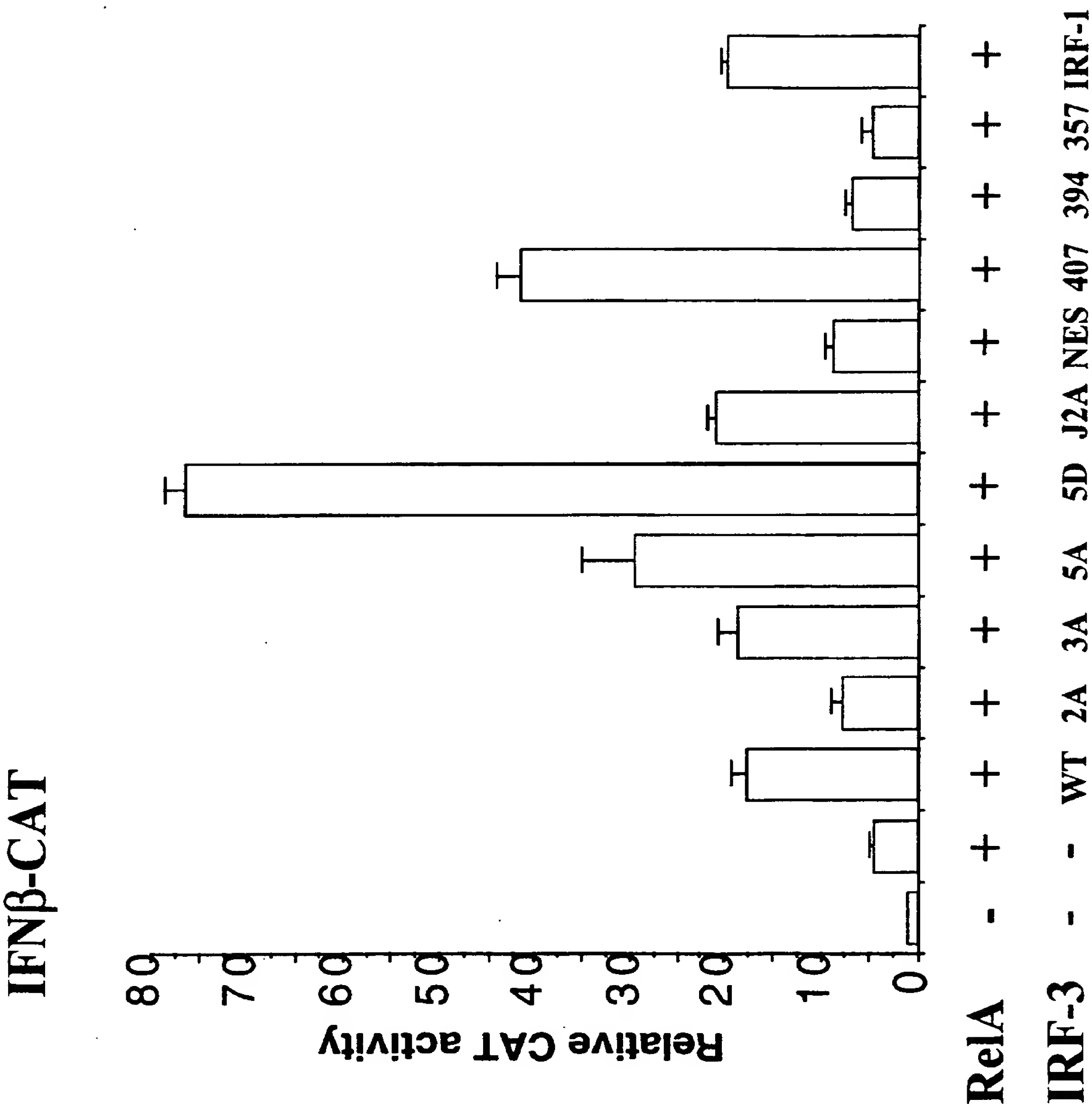
IFNβ-CAT
Relative CAT activity
0 10 20 30 40 50 60 70 80
RelA - + + + + + + + + + + +
IRF-3 - - WT 2A 3A 5A 5D J2A NES 407 394 357 IRF-1

FIG. 6B

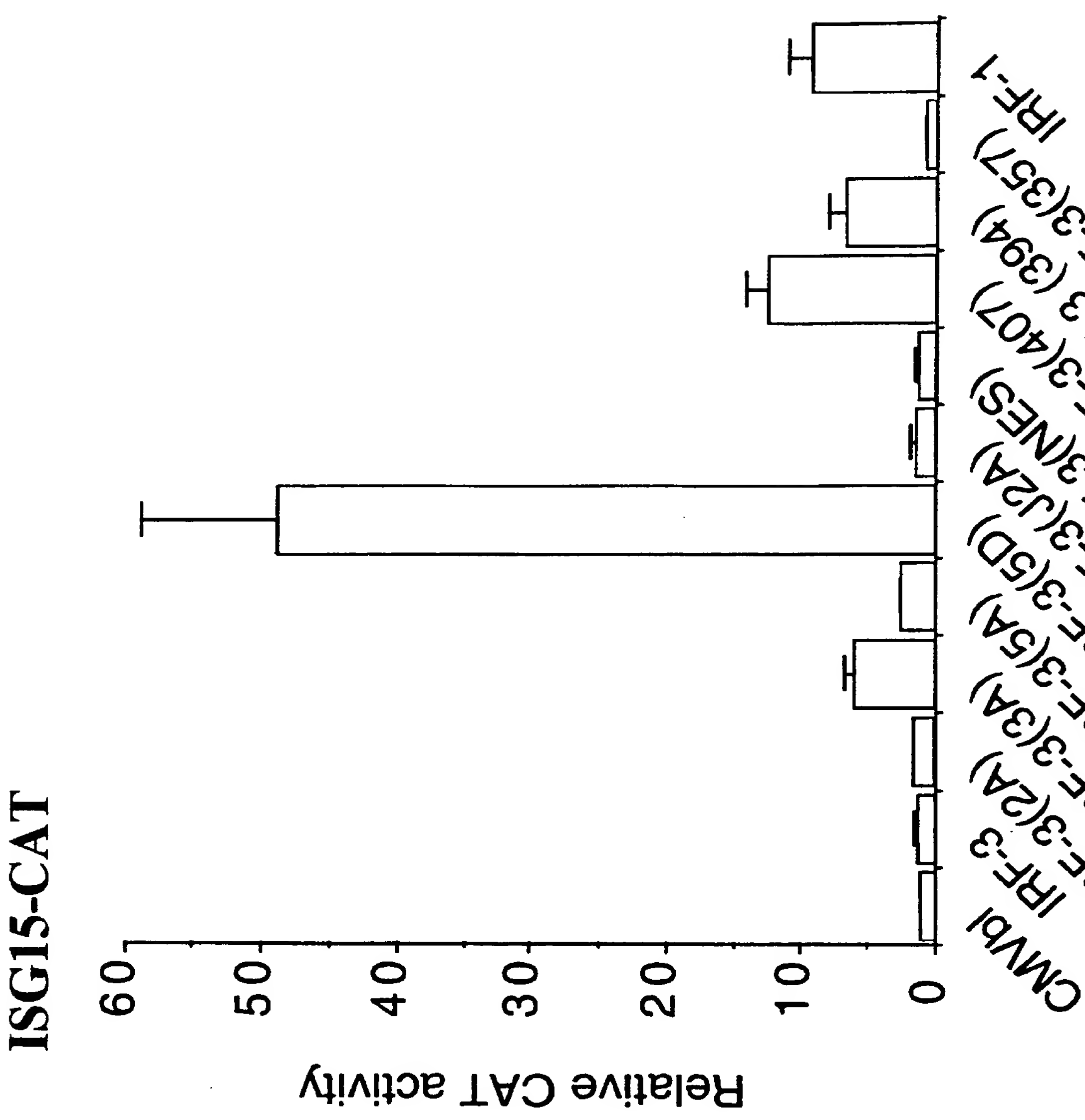
ISG15-CAT
Relative CAT activity
0
10
20
30
40
50
60
CMVbl
IRF-3
IRF-3(2A)
IRF-3(3A)
IRF-3(5A)
IRF-3(5D)
IRF-3(J2A)
IRF-3(NES)
IRF-3(407)
IRF-3 (394)
IRF-3(357)
IRF-1

FIG. 6C

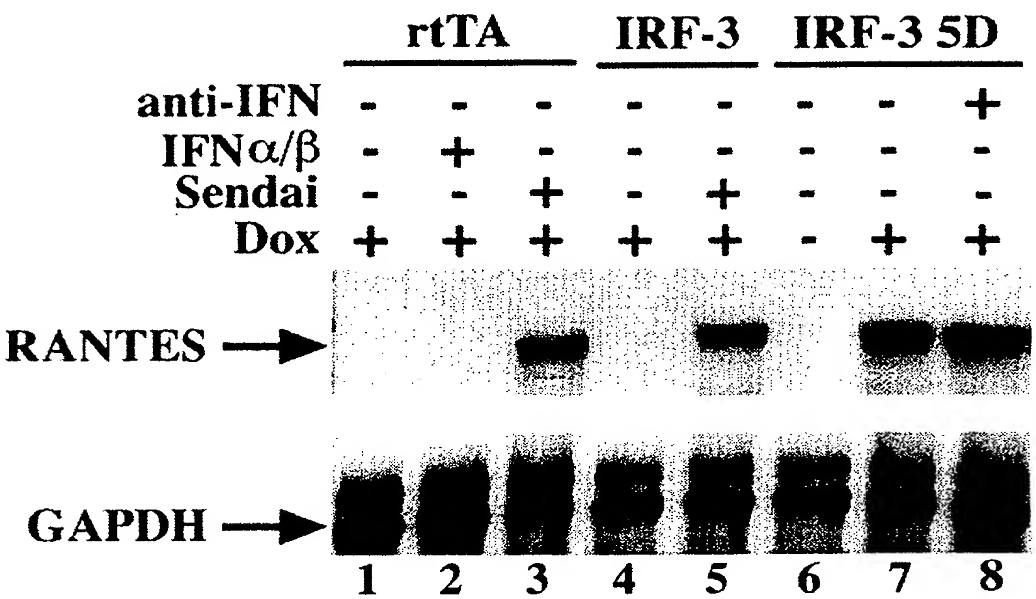
rtTA
IRF-3
IRF-3 5D
anti-IFN - - - - - - - +
IFNα/β - + - - - - - -
Sendai - - + - + - - -
Dox + + + + + - + +
RANTES
GAPDH
1 2 3 4 5 6 7 8

FIG. 7A

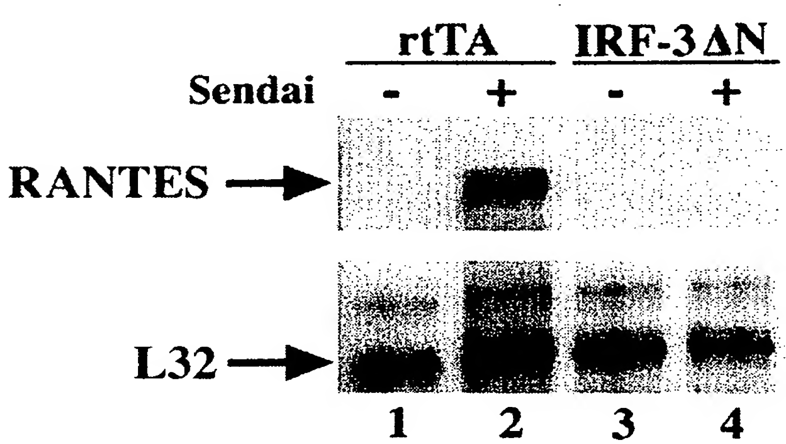
rtTA
IRF-3ΔN
Sendai - + - +
RANTES
L32
1 2 3 4

FIG. 7B

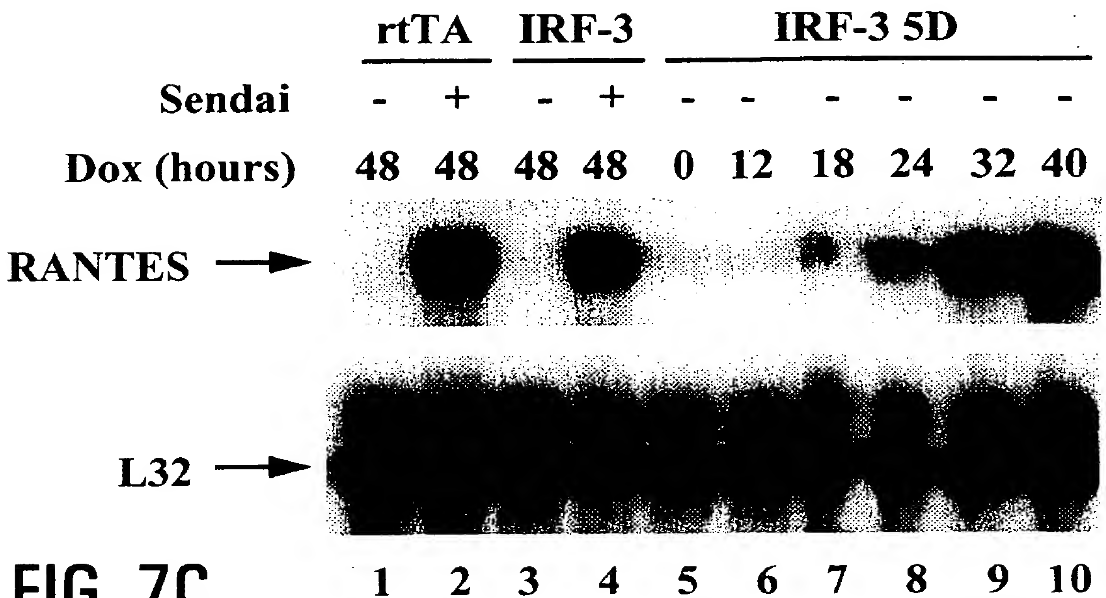
rtTA
IRF-3
IRF-3 5D
Sendai - + - + - - - - - -
Dox (hours) 48 48 48 48 0 12 18 24 32 40
RANTES
L32
1 2 3 4 5 6 7 8 9 10

FIG. 7C

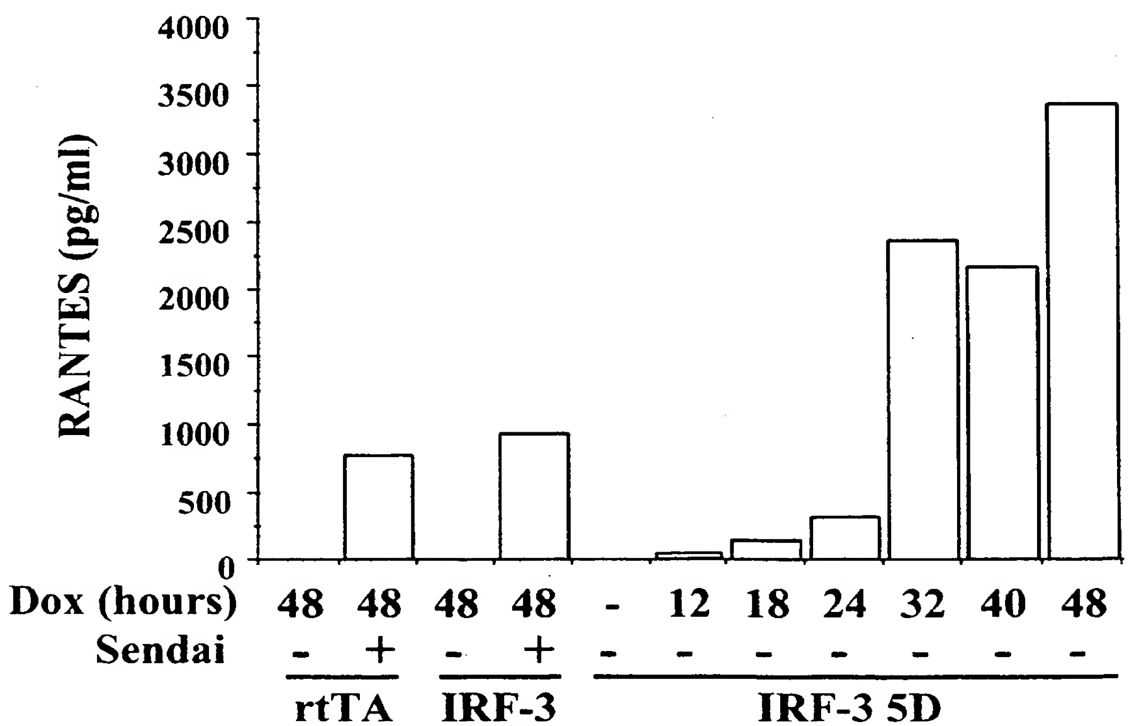
RANTES (pg/ml)
4000
3500
3000
2500
2000
1500
1000
500
0
Dox (hours) 48 48 48 48 - 12 18 24 32 40 48
Sendai - + - + - - - - - - -
rtTA
IRF-3
IRF-3 5D

FIG. 7D

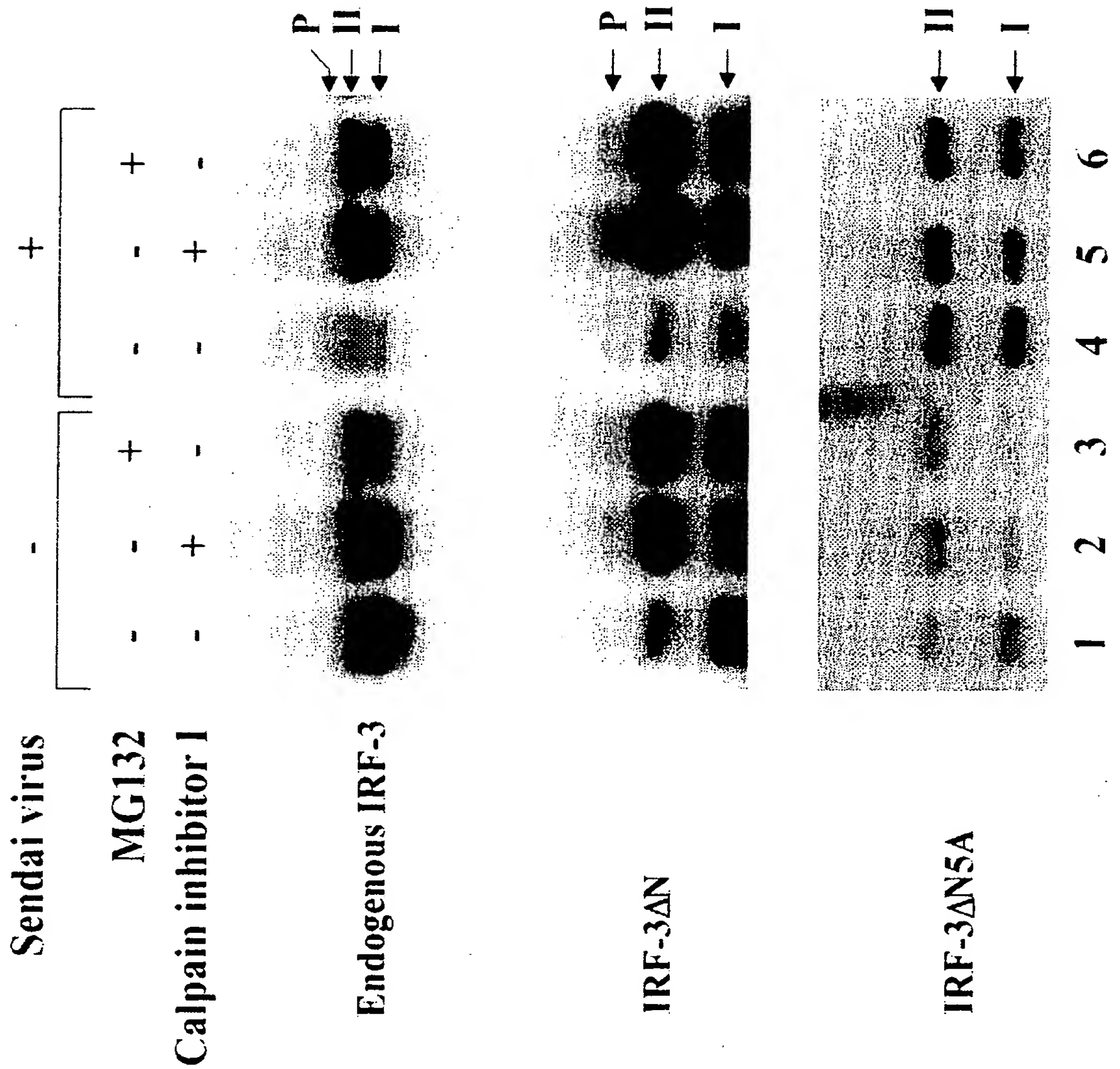

FIG. 8A

FIG. 8B

FIG. 8C

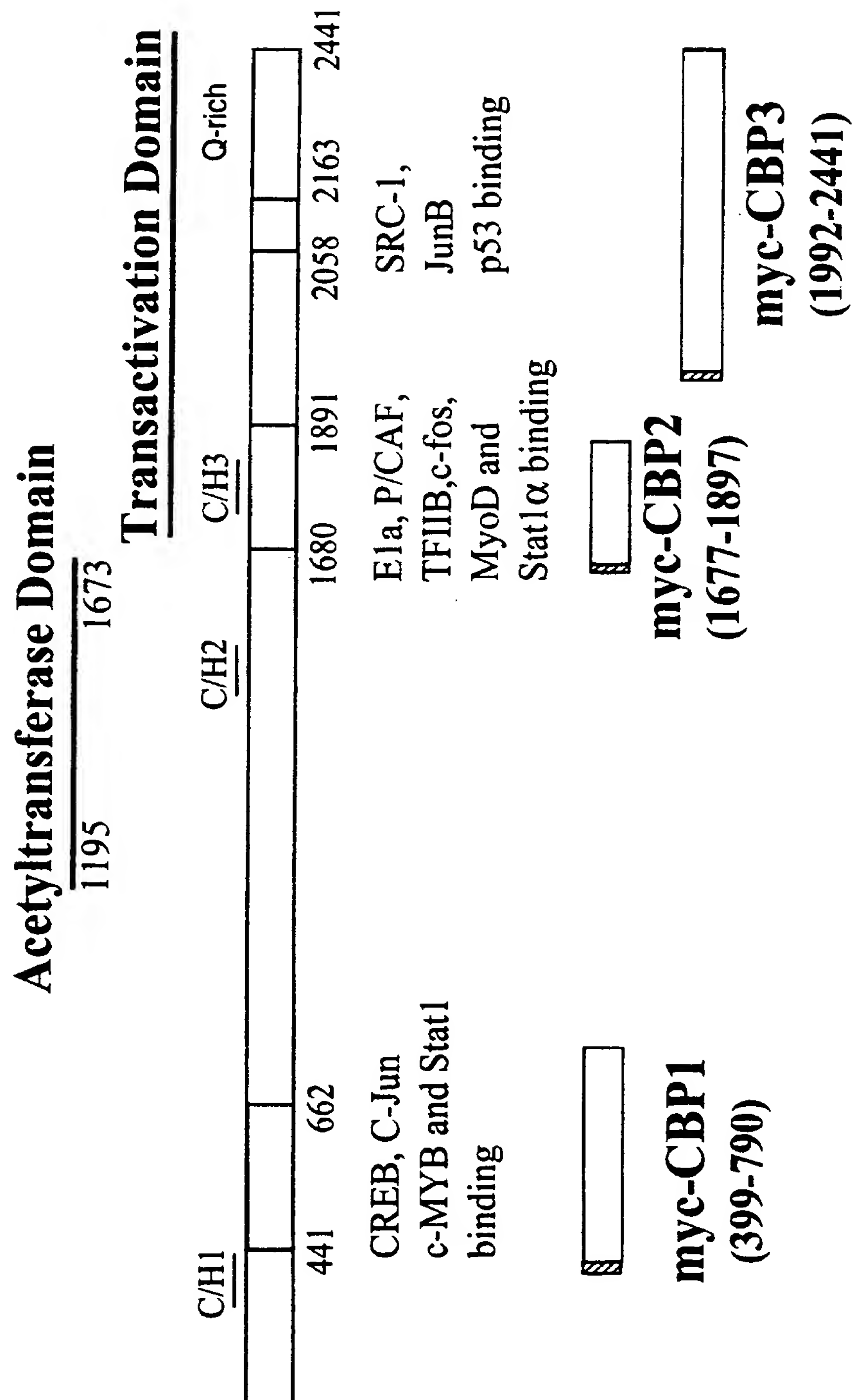
Acetyltransferase Domain
1195
1673
Transactivation Domain
C/H1
C/H2
C/H3
Q-rich
1
441
662
1680
1891
2058
2163
2441
Nuclear receptor binding
CREB, C-Jun c-MYB and Stat1 binding
E1a, P/CAF, TFIIB,c-fos, MyoD and Stat1α binding
SRC-1, JunB p53 binding
myc-CBP1 (399-790)
myc-CBP2 (1677-1897)
myc-CBP3 (1992-2441)

FIG. 9A

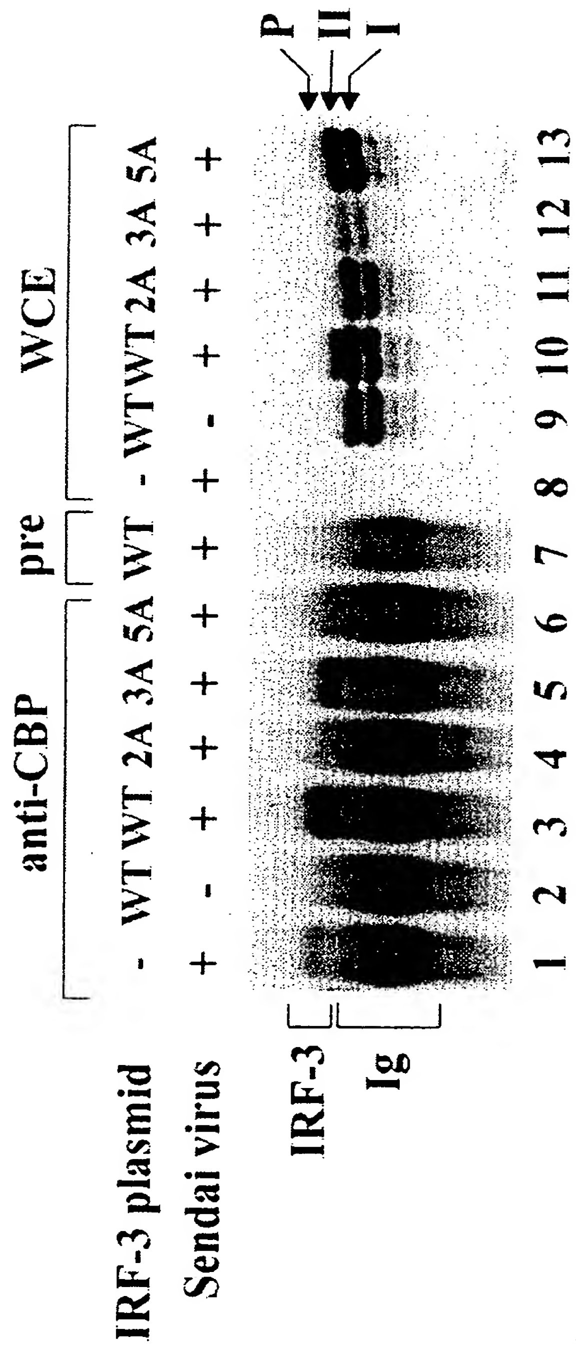
anti-CBP
pre
WCE
IRF-3 plasmid
- WT WT 2A 3A 5A WT - WT WT 2A 3A 5A
Sendai virus
+ - + + + + + + - + + + +
IRF-3
Ig
P
II
I
1 2 3 4 5 6 7 8 9 10 11 12 13

FIG. 9B

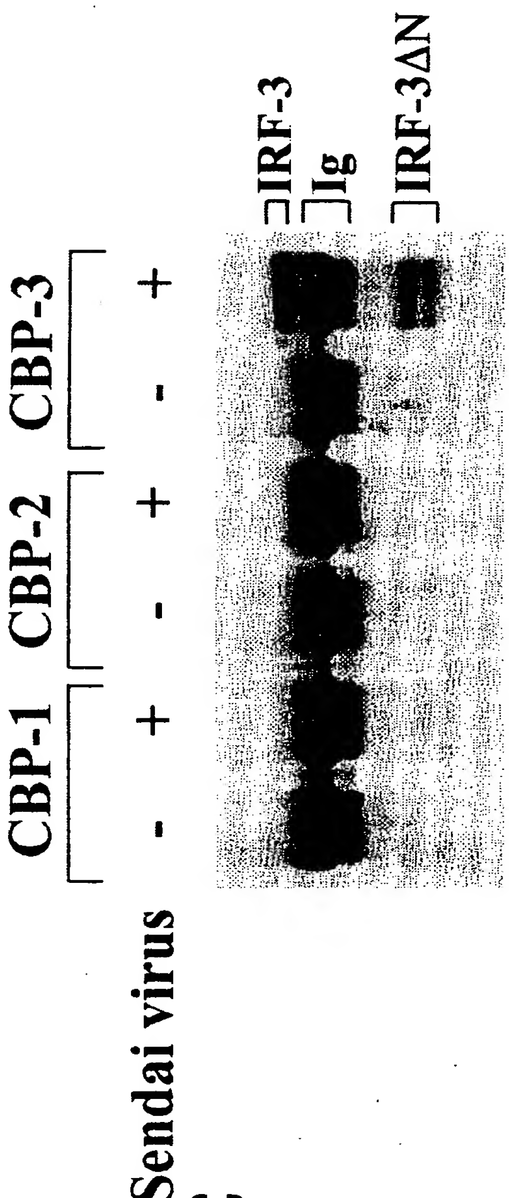
Sendai virus
CBP-1
CBP-2
CBP-3
- + - + - +
IRF-3
Ig
IRF-3ΔN

FIG. 9C


CBP-3
CBP-2
CBP-1
1 2 3 4 5 6

FIG. 9D

```
                10         20         30         40
                 *          *          *          *
ATG GGA ACC CCA AAG CCA CGG ATC CTG CCC TGG CTG GTG TCG CAG CTG
TAC CCT TGG GGT TTC GGT GCC TAG GAC GGG ACC GAC CAC AGC GTC GAC
 M   G   T   P   K   P   R   I   L   P   W   L   V   S   Q   L>

50         60         70         80         90
 *          *          *          *          *
GAC CTG GGG CAA CTG GAG GGC GTG GCC TGG GTG AAC AAG AGC CGC ACG
CTG GAC CCC GTT GAC CTC CCG CAC CGG ACC CAC TTG TTC TCG GCG TGC
 D   L   G   Q   L   E   G   V   A   W   V   N   K   S   R   T>

 100        110        120        130        140
   *          *          *          *          *
CGC TTC CGC ATC CCT TGG AAG CAC GGC CTA CGG CAG GAT GCA CAG CAG
GCG AAG GCG TAG GGA ACC TTC GTG CCG GAT GCC GTC CTA CGT GTC GTC
 R   F   R   I   P   W   K   H   G   L   R   Q   D   A   Q   Q>

   150        160        170        180        190
     *          *          *          *          *
GAG GAT TTC GGA ATC TTC CAG GCC TGG GCC GAG GCC ACT GGT GCA TAT
CTC CTA AAG CCT TAG AAG GTC CGG ACC CGG CTC CGG TGA CCA CGT ATA
 E   D   F   G   I   F   Q   A   W   A   E   A   T   G   A   Y>

      200        210        220        230        240
        *          *          *          *          *
GTT CCC GGG AGG GAT AAG CCA GAC CTG CCA ACC TGG AAG AGG AAT TTC
CAA GGG CCC TCC CTA TTC GGT CTG GAC GGT TGG ACC TTC TCC TTA AAG
 V   P   G   R   D   K   P   D   L   P   T   W   K   R   N   F>

         250        260        270        280
           *          *          *          *
CGC TCT GCC CTC AAC CGC AAA GAA GGG TTG CGT TTA GCA GAG GAC CGG
GCG AGA CGG GAG TTG GCG TTT CTT CCC AAC GCA AAT CGT CTC CTG GCC
 R   S   A   L   N   R   K   E   G   L   R   L   A   E   D   R>

290        300        310        320        330
 *          *          *          *          *
AGC AAG GAC CCT CAC GAC CCA CAT AAA ATC TAC GAG TTT GTG AAC TCA
TCG TTC CTG GGA GTG CTG GGT GTA TTT TAG ATG CTC AAA CAC TTG AGT
 S   K   D   P   H   D   P   H   K   I   Y   E   F   V   N   S>

  340        350        360        370        380
    *          *          *          *          *
GGA GTT GGG GAC TTT TCC CAG CCA GAC ACC TCT CCG GAC ACC AAT GGT
CCT CAA CCC CTG AAA AGG GTC GGT CTG TGG AGA GGC CTG TGG TTA CCA
 G   V   G   D   F   S   Q   P   D   T   S   P   D   T   N   G>

     390        400        410        420        430
       *          *          *          *          *
GGA GGC AGT ACT TCT GAT ACC CAG GAA GAC ATT CTG GAT GAG TTA CTG
CCT CCG TCA TGA AGA CTA TGG GTC CTT CTG TAA GAC CTA CTC AAT GAC
 G   G   S   T   S   D   T   Q   E   D   I   L   D   E   L   L>
```

FIG. 10

```
          440         450         460         470         480
            *           *           *           *           *
GGT AAC ATG GTG TTG GCC CCA CTC CCA GAT CCG GGA CCC CCA AGC CTG
CCA TTG TAC CAC AAC CGG GGT GAG GGT CTA GGC CCT GGG GGT TCG GAC
 G   N   M   V   L   A   P   L   P   D   P   G   P   P   S   L>

              490         500         510         520
                *           *           *           *
GCT GTA GCC CCT GAG CCC TGC CCT CAG CCC CTG CGG AGC CCC AGC TTG
CGA CAT CGG GGA CTC GGG ACG GGA GTC GGG GAC GCC TCG GGG TCG AAC
 A   V   A   P   E   P   C   P   Q   P   L   R   S   P   S   L>

530         540         550         560         570
  *           *           *           *           *
GAC AAT CCC ACT CCC TTC CCA AAC CTG GGG CCC TCT GAG AAC CCA CTG
CTG TTA GGG TGA GGG AAG GGT TTG GAC CCC GGG AGA CTC TTG GGT GAC
 D   N   P   T   P   F   P   N   L   G   P   S   E   N   P   L>

   580         590         600         610         620
     *           *           *           *           *
AAG CGG CTG TTG GTG CCG GGG GAA GAG TGG GAG TTC GAG GTG ACA GCC
TTC GCC GAC AAC CAC GGC CCC CTT CTC ACC CTC AAG CTC CAC TGT CGG
 K   R   L   L   V   P   G   E   E   W   E   F   E   V   T   A>

      630         640         650         660         670
        *           *           *           *           *
TTC TAC CGG GGC CGC CAA GTC TTC CAG CAG ACC ATC TCC TGC CCG GAG
AAG ATG GCC CCG GCG GTT CAG AAG GTC GTC TGG TAG AGG ACG GGC CTC
 F   Y   R   G   R   Q   V   F   Q   Q   T   I   S   C   P   E>

         680         690         700         710         720
           *           *           *           *           *
GGC CTG CGG CTG GTG GGG TCC GAA GTG GGA GAC AGG ACG CTG CCT GGA
CCG GAC GCC GAC CAC CCC AGG CTT CAC CCT CTG TCC TGC GAC GGA CCT
 G   L   R   L   V   G   S   E   V   G   D   R   T   L   P   G>

             730         740         750         760
               *           *           *           *
TGG CCA GTC ACA CTG CCA GAC CCT GGC ATG TCC CTG ACA GAC AGG GGA
ACC GGT CAG TGT GAC GGT CTG GGA CCG TAC AGG GAC TGT CTG TCC CCT
 W   P   V   T   L   P   D   P   G   M   S   L   T   D   R   G>

770         780         790         800         810
  *           *           *           *           *
GTG ATG AGC TAC GTG AGG CAT GTG CTG AGC TGC CTG GGT GGG GGA CTG
CAC TAC TCG ATG CAC TCC GTA CAC GAC TCG ACG GAC CCA CCC CCT GAC
 V   M   S   Y   V   R   H   V   L   S   C   L   G   G   G   L>

   820         830         840         850         860
     *           *           *           *           *
GCT CTC TGG CGG GCC GGG CAG TGG CTC TGG GCC CAG CGG CTG GGG CAC
CGA GAG ACC GCC CGG CCC GTC ACC GAG ACC CGG GTC GCC GAC CCC GTG
 A   L   W   R   A   G   Q   W   L   W   A   Q   R   L   G   H>

      870         880         890         900         910
        *           *           *           *           *
TGC CAC ACA TAC TGG GCA GTG AGC GAG GAG CTG CTC CCC AAC AGC GGG
ACG GTG TGT ATG ACC CGT CAC TCG CTC CTC GAC GAG GGG TTG TCG CCC
 C   H   T   Y   W   A   V   S   E   E   L   L   P   N   S   G>
```

FIG. 10
CONTINUED

```
            920          930          940          950          960
              *            *            *            *            *
 CAT GGG CCT GAT GGC GAG GTC CCC AAG GAC AAG GAA GGA GGC GTG TTT
 GTA CCC GGA CTA CCG CTC CAG GGG TTC CTG TTC CTT CCT CCG CAC AAA
  H   G   P   D   G   E   V   P   K   D   K   E   G   G   V   F>

               970          980          990         1000
                 *            *            *            *
 GAC CTG GGG CCC TTC ATT GTA GAT CTG ATT ACC TTC ACG GAA GGA AGC
 CTG GAC CCC GGG AAG TAA CAT CTA GAC TAA TGG AAG TGC CTT CCT TCG
  D   L   G   P   F   I   V   D   L   I   T   F   T   E   G   S>

1010         1020         1030         1040         1050
  *            *            *            *            *
 GGA CGC TCA CCA CGC TAT GCC CTC TGG TTC TGT GTG GGG GAG TCA TGG
 CCT GCG AGT GGT GCG ATA CGG GAG ACC AAG ACA CAC CCC CTC AGT ACC
  G   R   S   P   R   Y   A   L   W   F   C   V   G   E   S   W>

    1060         1070         1080         1090         1100
       *            *            *            *            *
 CCC CAG GAC CAG CCG TGG ACC AAG AGG CTC GTG ATG GTC AAG GTT GTG
 GGG GTC CTG GTC GGC ACC TGG TTC TCC GAG CAC TAC CAG TTC CAA CAC
  P   Q   D   Q   P   W   T   K   R   L   V   M   V   K   V   V>

      1110         1120         1130         1140         1150
         *            *            *            *            *
 CCC ACG TGC CTC AGG GCC TTG GTA GAA ATG GCC CGG GTA GGG GGT GCC
 GGG TGC ACG GAG TCC CGG AAC CAT CTT TAC CGG GCC CAT CCC CCA CGG
  P   T   C   L   R   A   L   V   E   M   A   R   V   G   G   A>

          1160         1170         1180         1190         1200
             *            *            *            *            *
 TCC TCC CTG GAG AAT ACT GTG GAC CTG CAC ATT GAC AAC GAC CAC CCA
 AGG AGG GAC CTC TTA TGA CAC CTG GAC GTG TAA CTG TTG CTG GTG GGT
  S   S   L   E   N   T   V   D   L   H   I   D   N   D   H   P>

              1210         1220         1230         1240
                 *            *            *            *
 CTC GAC CTC GAC GAC GAC CAG TAC AAG GCC TAC CTG CAG GAC TTG GTG
 GAG CTG GAG CTG CTG CTG GTC ATG TTC CGG ATG GAC GTC CTG AAC CAC
  L   D   L   D   D   D   Q   Y   K   A   Y   L   Q   D   L   V>

1250         1260         1270         1280
  *            *            *            *
 GAG GGC ATG GAT TTC CAG GGC CCT GGG GAG AGC TGA
 CTC CCG TAC CTA AAG GTC CCG GGA CCC CTC TCG ACT
  E   G   M   D   F   Q   G   P   G   E   S>
```

FIG. 10
CONTINUED

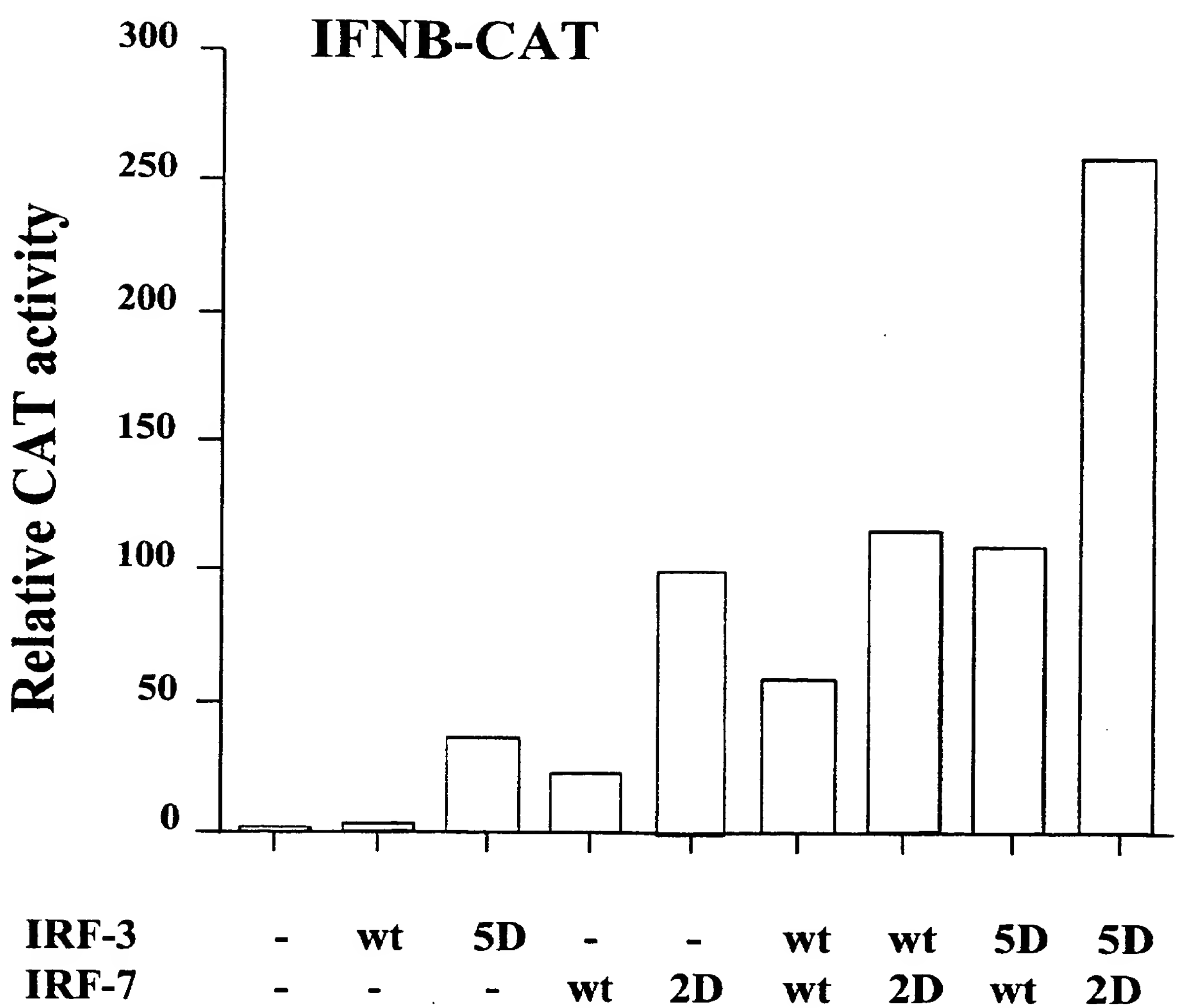
IFNB-CAT
Relative CAT activity
300
250
200
150
100
50
0
IRF-3 - wt 5D - - wt wt 5D 5D
IRF-7 - - - wt 2D wt 2D wt 2D

FIG. 11

```
              10                  20                  30                  40
               *                   *                   *                   *
ATG GCC TTG GCT CCT GAG AGG GCA GCC CCA CGC GTG CTG TTC GGA GAG
TAC CGG AAC CGA GGA CTC TCC CGT CGG GGT GCG CAC GAC AAG CCT CTC
 M   A   L   A   P   E   R   A   A   P   R   V   L   F   G   E>

50                  60                  70                  80                  90
 *                   *                   *                   *                   *
TGG CTC CTT GGA GAG ATC AGC AGC GGC TGC TAT GAG GGG CTG CAG TGG
ACC GAG GAA CCT CTC TAG TCG TCG CCG ACG ATA CTC CCC GAC GTC ACC
 W   L   L   G   E   I   S   S   G   C   Y   E   G   L   Q   W>

   100                 110                 120                 130                 140
     *                   *                   *                   *                   *
CTG GAC GAG GCC CGC ACC TGT TTC CGC GTG CCC TGG AAG CAC TTC GCG
GAC CTG CTC CGG GCG TGG ACA AAG GCG CAC GGG ACC TTC GTG AAG CGC
 L   D   E   A   R   T   C   F   R   V   P   W   K   H   F   A>

      150                 160                 170                 180                 190
        *                   *                   *                   *                   *
CGC AAG GAC CTG AGC GAG GCC GAC GCG CGC ATC TTC AAG GCC TGG GCT
GCG TTC CTG GAC TCG CTC CGG CTG CGC GCG TAG AAG TTC CGG ACC CGA
 R   K   D   L   S   E   A   D   A   R   I   F   K   A   W   A>

         200                 210                 220                 230                 240
           *                   *                   *                   *                   *
GTG GCC CGC GGC AGG TGG CCG CCT AGC AGC AGG GGA GGT GGC CCG CCC
CAC CGG GCG CCG TCC ACC GGC GGA TCG TCG TCC CCT CCA CCG GGC GGG
 V   A   R   G   R   W   P   P   S   S   R   G   G   G   P   P>

              250                 260                 270                 280
                *                   *                   *                   *
CCC GAG GCT GAG ACT GCG GAG CGC GCC GGC TGG AAA ACC AAC TTC CGC
GGG CTC CGA CTC TGA CGC CTC GCG CGG CCG ACC TTT TGG TTG AAG GCG
 P   E   A   E   T   A   E   R   A   G   W   K   T   N   F   R>

290                 300                 310                 320                 330
 *                   *                   *                   *                   *
TGC GCA CTG CGC AGC ACG CGT CGC TTC GTG ATG CTG CGG GAT AAC TCG
ACG CGT GAC GCG TCG TGC GCA GCG AAG CAC TAC GAC GCC CTA TTG AGC
 C   A   L   R   S   T   R   R   F   V   M   L   R   D   N   S>

   340                 350                 360                 370                 380
     *                   *                   *                   *                   *
GGG GAC CCG GCC GAC CCG CAC AAG GTG TAC GCG CTC AGC CGG GAG CTG
CCC CTG GGC CGG CTG GGC GTG TTC CAC ATG CGC GAG TCG GCC CTC GAC
 G   D   P   A   D   P   H   K   V   Y   A   L   S   R   E   L>
```

FIG. 12

22/30

```
     390             400            410            420              430
       *               *              *              *                *
TGC TGG CGA GAA GGC CCA GGC ACG GAC CAG ACT GAG GCA GAG GCC CCC
ACG ACC GCT CTT CCG GGT CCG TGC CTG GTC TGA CTC CGT CTC CGG GGG
 C   W   R   E   G   P   G   T   D   Q   T   E   A   E   A   P>

        440            450             460            470            480
          *              *               *              *              *
GCA GCT GTC CCA CCA CCA CAG GGT GGG CCC CCA GGG CCA TTC TTG GCA
CGT CGA CAG GGT GGT GGT GTC CCA CCC GGG GGT CCC GGT AAG AAC CGT
 A   A   V   P   P   P   Q   G   G   P   P   G   P   F   L   A>

           490            500            510             520
             *              *              *               *
CAC ACA CAT GCT GGA CTC CAA GCC CCA GGC CCC CTC CCT GCC CCA GCT
GTG TGT GTA CGA CCT GAG GTT CGG GGT CCG GGG GAG GGA CGG GGT CGA
 H   T   H   A   G   L   Q   A   P   G   P   L   P   A   P   A>

530            540             550            560            570
  *              *               *              *              *
GGT GAC AAG GGG GAC CTC CTG CTC CAG GCA GTG CAA CAG AGC TGC CTG
CCA CTG TTC CCC CTG GAG GAC GAG GTC CGT CAC GTT GTC TCG ACG GAC
 G   D   K   G   D   L   L   L   Q   A   V   Q   Q   S   C   L>

   580            590            600             610            620
     *              *              *               *              *
GCA GAC CAT CTG CTG ACA GCG TCA TGG GGG GCA GAT CCA GTC CCA ACC
CGT CTG GTA GAC GAC TGT CGC AGT ACC CCC CGT CTA GGT CAG GGT TGG
 A   D   H   L   L   T   A   S   W   G   A   D   P   V   P   T>

     630            640             650            660              670
       *              *               *              *                *
AAG GCT CCT GGA GAG GGA CAA GAA GGG CTT CCC CTG ACT GGG GCC TGT
TTC CGA GGA CCT CTC CCT GTT CTT CCC GAA GGG GAC TGA CCC CGG ACA
 K   A   P   G   E   G   Q   E   G   L   P   L   T   G   A   C>

        680            690             700            710            720
          *              *               *              *              *
GCT GGA GGC CCA GGG CTC CCT GCT GGG GAG CTG TAC GGG TGG GCA GTA
CGA CCT CCG GGT CCC GAG GGA CGA CCC CTC GAC ATG CCC ACC CGT CAT
 A   G   G   P   G   L   P   A   G   E   L   Y   G   W   A   V>

           730            740            750             760
             *              *              *               *
GAG ACG ACC CCC AGC CCC GGG CCC CAG CCC GCG GCA CTA ACG ACA GGC
CTC TGC TGG GGG TCG GGG CCC GGG GTC GGG CGC CGT GAT TGC TGT CCG
 E   T   T   P   S   P   G   P   Q   P   A   A   L   T   T   G>
```

FIG. 12
CONTINUED

```
770          780          790          800          810
 *            *            *            *            *
GAG GCC GCG GCC CCA GAG TCC CCG CAC CAG GCA GAG CCG TAC CTG TCA
CTC CGG CGC CGG GGT CTC AGG GGC GTG GTC CGT CTC GGC ATG GAC AGT
 E   A   A   A   P   E   S   P   H   Q   A   E   P   Y   L   S>

  820          830          840          850          860
   *            *            *            *            *
CCC TCC CCA AGC GCC TGC ACC GCG GTG CAA GAG CCC AGC CCA GGG GCG
GGG AGG GGT TCG CGG ACG TGG CGC CAC GTT CTC GGG TCG GGT CCC CGC
 P   S   P   S   A   C   T   A   V   Q   E   P   S   P   G   A>

    870          880          890          900          910
     *            *            *            *            *
CTG GAC GTG ACC ATC ATG TAC AAG GGC CGC ACG GTG CTG CAG AAG GTG
GAC CTG CAC TGG TAG TAC ATG TTC CCG GCG TGC CAC GAC GTC TTC CAC
 L   D   V   T   I   M   Y   K   G   R   T   V   L   Q   K   V>

        920          930          940          950          960
         *            *            *            *            *
GTG GGA CAC CCG AGC TGC ACG TTC CTA TAC GGC CCC CCA GAC CCA GCT
CAC CCT GTG GGC TCG ACG TGC AAG GAT ATG CCG GGG GGT CTG GGT CGA
 V   G   H   P   S   C   T   F   L   Y   G   P   P   D   P   A>

           970          980          990         1000
            *            *            *            *
GTC CGG GCC ACA GAC CCC CAG CAG GTA GCA TTC CCC AGC CCT GCC GAG
CAG GCC CGG TGT CTG GGG GTC GTC CAT CGT AAG GGG TCG GGA CGG CTC
 V   R   A   T   D   P   Q   Q   V   A   F   P   S   P   A   E>

1010         1020         1030         1040         1050
 *            *            *            *            *
CTC CCG GAC CAG AAG CAG CTG CGC TAC ACG GAG GAA CTG CTG CGG CAC
GAG GGC CTG GTC TTC GTC GAC GCG ATG TGC CTC CTT GAC GAC GCC GTG
 L   P   D   Q   K   Q   L   R   Y   T   E   E   L   L   R   H>

  1060         1070         1080         1090         1100
   *            *            *            *            *
GTG GCC CCT GGG TTG CAC CTG GAG CTT CGG GGG CCA CAG CTG TGG GCC
CAC CGG GGA CCC AAC GTG GAC CTC GAA GCC CCC GGT GTC GAC ACC CGG
 V   A   P   G   L   H   L   E   L   R   G   P   Q   L   W   A>

    1110         1120         1130         1140         1150
     *            *            *            *            *
CGG CGC ATG GGC AAG TGC AAG GTG TAC TGG GAG GTG GGC GGA CCC CCA
GCC GCG TAC CCG TTC ACG TTC CAC ATG ACC CTC CAC CCG CCT GGG GGT
 R   R   M   G   K   C   K   V   Y   W   E   V   G   G   P   P>
```

FIG. 12
CONTINUED

```
       1160        1170         1180        1190        1200
          *           *            *           *           *
GGC TCC GCC AGC CCC TCC ACC CCA GCC TGC CTG CTG CCT CGG AAC TGT
CCG AGG CGG TCG GGG AGG TGG GGT CGG ACG GAC GAC GGA GCC TTG ACA
 G   S   A   S   P   S   T   P   A   C   L   L   P   R   N   C>

          1210        1220        1230        1240
             *           *           *           *
GAC ACC CCC ATC TTC GAC TTC AGA GTC TTC TTC CAA GAG CTG GTG GAA
CTG TGG GGG TAG AAG CTG AAG TCT CAG AAG AAG GTT CTC GAC CAC CTT
 D   T   P   I   F   D   F   R   V   F   F   Q   E   L   V   E>

1250        1260         1270        1280        1290
  *           *            *           *           *
TTC CGG GCA CGG CAG CGC CGT GGC TCC CCA CGC TAT ACC ATC TAC CTG
AAG GCC CGT GCC GTC GCG GCA CCG AGG GGT GCG ATA TGG TAG ATG GAC
 F   R   A   R   Q   R   R   G   S   P   R   Y   T   I   Y   L>

  1300        1310        1320         1330        1340
     *           *           *            *           *
GGC TTC GGG CAG GAC CTG TCA GCT GGG AGG CCC AAG GAG AAG AGC CTG
CCG AAG CCC GTC CTG GAC AGT CGA CCC TCC GGG TTC CTC TTC TCG GAC
 G   F   G   Q   D   L   S   A   G   R   P   K   E   K   S   L>

    1350         1360        1370        1380         1390
       *            *           *           *            *
GTC CTG GTG AAG CTG GAA CCC TGG CTG TGC CGA GTG CAC CTA GAG GGC
CAG GAC CAC TTC GAC CTT GGG ACC GAC ACG GCT CAC GTG GAT CTC CCG
 V   L   V   K   L   E   P   W   L   C   R   V   H   L   E   G>

       1400        1410         1420        1430        1440
          *           *            *           *           *
ACG CAG CGT GAG GGT GTG TCT TCC CTG GAT AGC AGC GAC CTC GAC CTC
TGC GTC GCA CTC CCA CAC AGA AGG GAC CTA TCG TCG CTG GAG CTG GAG
 T   Q   R   E   G   V   S   S   L   D   S   S   D   L   D   L>

          1450        1460        1470        1480
             *           *           *           *
TGC CTG TCC AGC GCC AAC AGC CTC TAT GAC GAC ATC GAG TGC TTC CTT
ACG GAC AGG TCG CGG TTG TCG GAG ATA CTG CTG TAG CTC ACG AAG GAA
 C   L   S   S   A   N   S   L   Y   D   D   I   E   C   F   L>

1490        1500         1510
  *           *            *
ATG GAG CTG GAG CAG CCC GCC TAG
TAC CTC GAC CTC GTC GGG CGG ATC
 M   E   L   E   Q   P   A>
```

FIG. 12
CONTINUED

```
              10         20         30         40
               *          *          *          *
ATG GCC TTG GCT CCT GAG AGG GCA GCC CCA CGC GTG CTG TTC GGA GAG
TAC CGG AAC CGA GGA CTC TCC CGT CGG GGT GCG CAC GAC AAG CCT CTC
 M   A   L   A   P   E   R   A   A   P   R   V   L   F   G   E>

50         60         70         80         90
 *          *          *          *          *
TGG CTC CTT GGA GAG ATC AGC AGC GGC TGC TAT GAG GGG CTG CAG TGG
ACC GAG GAA CCT CTC TAG TCG TCG CCG ACG ATA CTC CCC GAC GTC ACC
 W   L   L   G   E   I   S   S   G   C   Y   E   G   L   Q   W>

  100        110        120        130        140
    *          *          *          *          *
CTG GAC GAG GCC CGC ACC TGT TTC CGC GTG CCC TGG AAG CAC TTC GCG
GAC CTG CTC CGG GCG TGG ACA AAG GCG CAC GGG ACC TTC GTG AAG CGC
 L   D   E   A   R   T   C   F   R   V   P   W   K   H   F   A>

    150        160        170        180        190
      *          *          *          *          *
CGC AAG GAC CTG AGC GAG GCC GAC GCG CGC ATC TTC AAG GCC TGG GCT
GCG TTC CTG GAC TCG CTC CGG CTG CGC GCG TAG AAG TTC CGG ACC CGA
 R   K   D   L   S   E   A   D   A   R   I   F   K   A   W   A>

       200        210        220        230        240
         *          *          *          *          *
GTG GCC CGC GGC AGG TGG CCG CCT AGC AGC AGG GGA GGT GGC CCG CCC
CAC CGG GCG CCG TCC ACC GGC GGA TCG TCG TCC CCT CCA CCG GGC GGG
 V   A   R   G   R   W   P   P   S   S   R   G   G   G   P   P>

           250        260        270        280
             *          *          *          *
CCC GAG GCT GAG ACT GCG GAG CGC GCC GGC TGG AAA ACC AAC TTC CGC
GGG CTC CGA CTC TGA CGC CTC GCG CGG CCG ACC TTT TGG TTG AAG GCG
 P   E   A   E   T   A   E   R   A   G   W   K   T   N   F   R>

290        300        310        320        330
 *          *          *          *          *
TGC GCA CTG CGC AGC ACG CGT CGC TTC GTG ATG CTG CGG GAT AAC TCG
ACG CGT GAC GCG TCG TGC GCA GCG AAG CAC TAC GAC GCC CTA TTG AGC
 C   A   L   R   S   T   R   R   F   V   M   L   R   D   N   S>
```

FIG. 13

```
    340          350          360          370          380
      *            *            *            *            *
GGG GAC CCG GCC GAC CCG CAC AAG GTG TAC GCG CTC AGC CGG GAG CTG
CCC CTG GGC CGG CTG GGC GTG TTC CAC ATG CGC GAG TCG GCC CTC GAC
 G   D   P   A   D   P   H   K   V   Y   A   L   S   R   E   L>

      390          400          410          420          430
        *            *            *            *            *
TGC TGG CGA GAA GGC CCA GGC ACG GAC CAG ACT GAG GCA GAG GCC CCC
ACG ACC GCT CTT CCG GGT CCG TGC CTG GTC TGA CTC CGT CTC CGG GGG
 C   W   R   E   G   P   G   T   D   Q   T   E   A   E   A   P>

         440          450          460          470          480
           *            *            *            *            *
GCA GCT GTC CCA CCA CCA CAG GGT GGG CCC CCA GGG CCA TTC TTG GCA
CGT CGA CAG GGT GGT GGT GTC CCA CCC GGG GGT CCC GGT AAG AAC CGT
 A   A   V   P   P   P   Q   G   G   P   P   G   P   F   L   A>

            490          500          510          520
              *            *            *            *
CAC ACA CAT GCT GGA CTC CAA GCC CCA GGC CCC CTC CCT GCC CCA GCT
GTG TGT GTA CGA CCT GAG GTT CGG GGT CCG GGG GAG GGA CGG GGT CGA
 H   T   H   A   G   L   Q   A   P   G   P   L   P   A   P   A>

530          540          550          560          570
 *            *            *            *            *
GGT GAC AAG GGG GAC CTC CTG CTC CAG GCA GTG CAA CAG AGC TGC CTG
CCA CTG TTC CCC CTG GAG GAC GAG GTC CGT CAC GTT GTC TCG ACG GAC
 G   D   K   G   D   L   L   L   Q   A   V   Q   Q   S   C   L>

   580          590          600          610          620
     *            *            *            *            *
GCA GAC CAT CTG CTG ACA GCG TCA TGG GGG GCA GAT CCA GTC CCA ACC
CGT CTG GTA GAC GAC TGT CGC AGT ACC CCC CGT CTA GGT CAG GGT TGG
 A   D   H   L   L   T   A   S   W   G   A   D   P   V   P   T>

      630          640          650          660          670
        *            *            *            *            *
AAG GCT CCT GGA GAG GGA CAA GAA GGG CTT CCC CTG ACT GGG GCC TGT
TTC CGA GGA CCT CTC CCT GTT CTT CCC GAA GGG GAC TGA CCC CGG ACA
 K   A   P   G   E   G   Q   E   G   L   P   L   T   G   A   C>

         680          690          700          710          720
           *            *            *            *            *
GCT GGA GGC CCA GGG CTC CCT GCT GGG GAG CTG TAC GGG TGG GCA GTA
CGA CCT CCG GGT CCC GAG GGA CGA CCC CTC GAC ATG CCC ACC CGT CAT
 A   G   G   P   G   L   P   A   G   E   L   Y   G   W   A   V>
```

FIG. 13
CONTINUED

```
              730                 740                 750                   760
               *                   *                   *                     *
 GAG ACG ACC CCC AGC CCC ACT TCT GAT ACC CAG GAA GAC ATT CTG GAT
 CTC TGC TGG GGG TCG GGG TGA AGA CTA TGG GTC CTT CTG TAA GAC CTA
  E   T   T   P   S   P   T   S   D   T   Q   E   D   I   L   D>

770                780                  790               800                 810
 *                  *                    *                 *                   *
 GAG TTA CTG GGT AAC ATG GTG TTG GCC CCA CTC CCA GAT CCG GGA CCC
 CTC AAT GAC CCA TTG TAC CAC AAC CGG GGT GAG GGT CTA GGC CCT GGG
  E   L   L   G   N   M   V   L   A   P   L   P   D   P   G   P>

    820                 830                 840                 850                 860
     *                   *                   *                   *                   *
 CCA AGC CTG GCT GTA GCC CCT GAG CCC TGC CCT CAG CCC CTG CGG AGC
 GGT TCG GAC CGA CAT CGG GGA CTC GGG ACG GGA GTC GGG GAC GCC TCG
  P   S   L   A   V   A   P   E   P   C   P   Q   P   L   R   S>

       870                 880                 890                 900                 910
        *                   *                   *                   *                   *
 CCC AGC TTG GAC AAT CCC ACT CCC TTC CCA AAC CTG GGG CCC TCT GAG
 GGG TCG AAC CTG TTA GGG TGA GGG AAG GGT TTG GAC CCC GGG AGA CTC
  P   S   L   D   N   P   T   P   F   P   N   L   G   P   S   E>

           920                 930                 940                 950                 960
            *                   *                   *                   *                   *
 AAC CCA CTG AAG CGG CTG TTG GTG CCG GGG GAA GAG TGG GAG TTC GAG
 TTG GGT GAC TTC GCC GAC AAC CAC GGC CCC CTT CTC ACC CTC AAG CTC
  N   P   L   K   R   L   L   V   P   G   E   E   W   E   F   E>

              970                 980                 990                1000
               *                   *                   *                   *
 GTG ACA GCC TTC TAC CGG GGC CGC CAA GTC TTC CAG CAG ACC ATC TCC
 CAC TGT CGG AAG ATG GCC CCG GCG GTT CAG AAG GTC GTC TGG TAG AGG
  V   T   A   F   Y   R   G   R   Q   V   F   Q   Q   T   I   S>

1010                1020                1030                1040                1050
 *                   *                   *                   *                   *
 TGC CCG GAG GGC CTG CGG CTG GTG GGG TCC GAA GTG GGA GAC AGG ACG
 ACG GGC CTC CCG GAC GCC GAC CAC CCC AGG CTT CAC CCT CTG TCC TGC
  C   P   E   G   L   R   L   V   G   S   E   V   G   D   R   T>

    1060                1070                1080                1090                1100
     *                   *                   *                   *                   *
 CTG CCT GGA TGG CCA GTC ACA CTG CCA GAC CCT GGC ATG TCC CTG ACA
 GAC GGA CCT ACC GGT CAG TGT GAC GGT CTG GGA CCG TAC AGG GAC TGT
  L   P   G   W   P   V   T   L   P   D   P   G   M   S   L   T>
```

FIG. 13
CONTINUED

```
     1110         1120         1130         1140         1150
        *            *            *            *            *
GAC AGG GGA GTG ATG AGC TAC GTG AGG CAT GTG CTG AGC TGC CTG GGT
CTG TCC CCT CAC TAC TCG ATG CAC TCC GTA CAC GAC TCG ACG GAC CCA
 D   R   G   V   M   S   Y   V   R   H   V   L   S   C   L   G>

        1160         1170         1180         1190         1200
           *            *            *            *            *
GGG GGA CTG GCT CTC TGG CGG GCC GGG CAG TGG CTC TGG GCC CAG CGG
CCC CCT GAC CGA GAG ACC GCC CGG CCC GTC ACC GAG ACC CGG GTC GCC
 G   G   L   A   L   W   R   A   G   Q   W   L   W   A   Q   R>

           1210         1220         1230         1240
              *            *            *            *
CTG GGG CAC TGC CAC ACA TAC TGG GCA GTG AGC GAG GAG CTG CTC CCC
GAC CCC GTG ACG GTG TGT ATG ACC CGT CAC TCG CTC CTC GAC GAG GGG
 L   G   H   C   H   T   Y   W   A   V   S   E   E   L   L   P>

1250         1260         1270         1280         1290
 *            *            *            *            *
AAC AGC GGG CAT GGG CCT GAT GGC GAG GTC CCC AAG GAC AAG GAA GGA
TTG TCG CCC GTA CCC GGA CTA CCG CTC CAG GGG TTC CTG TTC CTT CCT
 N   S   G   H   G   P   D   G   E   V   P   K   D   K   E   G>

  1300         1310         1320         1330         1340
     *            *            *            *            *
GGC GTG TTT GAC CTG GGG CCC TTC ATT GTA GAT CTG ATT ACC TTC ACG
CCG CAC AAA CTG GAC CCC GGG AAG TAA CAT CTA GAC TAA TGG AAG TGC
 G   V   F   D   L   G   P   F   I   V   D   L   I   T   F   T>

     1350         1360         1370         1380         1390
        *            *            *            *            *
GAA GGA AGC GGA CGC TCA CCA CGC TAT GCC CTC TGG TTC TGT GTG GGG
CTT CCT TCG CCT GCG AGT GGT GCG ATA CGG GAG ACC AAG ACA CAC CCC
 E   G   S   G   R   S   P   R   Y   A   L   W   F   C   V   G>

        1400         1410         1420         1430         1440
           *            *            *            *            *
GAG TCA TGG CCC CAG GAC CAG CCG TGG ACC AAG AGG CTC GTG ATG GTC
CTC AGT ACC GGG GTC CTG GTC GGC ACC TGG TTC TCC GAG CAC TAC CAG
 E   S   W   P   Q   D   Q   P   W   T   K   R   L   V   M   V>

           1450         1460         1470         1480
              *            *            *            *
AAG GTT GTG CCC ACG TGC CTC AGG GCC TTG GTA GAA ATG GCC CGG GTA
TTC CAA CAC GGG TGC ACG GAG TCC CGG AAC CAT CTT TAC CGG GCC CAT
 K   V   V   P   T   C   L   R   A   L   V   E   M   A   R   V>

1490         1500         1510         1520         1530
 *            *            *            *            *
GGG GGT GCC TCC TCC CTG GAG AAT ACT GTG GAC CTG CAC ATT GAC AAC
CCC CCA CGG AGG AGG GAC CTC TTA TGA CAC CTG GAC GTG TAA CTG TTG
 G   G   A   S   S   L   E   N   T   V   D   L   H   I   D   N>
```

FIG. 13
CONTINUED

```
 1540          1550          1560          1570          1580
     *             *             *             *             *
GAC CAC CCA CTC GAC CTC GAC GAC GAC CAG TAC AAG GCC TAC CTG CAG
CTG GTG GGT GAG CTG GAG CTG CTG CTG GTC ATG TTC CGG ATG GAC GTC
 D   H   P   L   D   L   D   D   D   Q   Y   K   A   Y   L   Q>

   1590          1600          1610          1620
     *             *             *             *
GAC TTG GTG GAG GGC ATG GAT TTC CAG GGC CCT GGG GAG AGC TGA
CTG AAC CAC CTC CCG TAC CTA AAG GTC CCG GGA CCC CTC TCG ACT
 D   L   V   E   G   M   D   F   Q   G   P   G   E   S>
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FIG. 13
CONTINUED

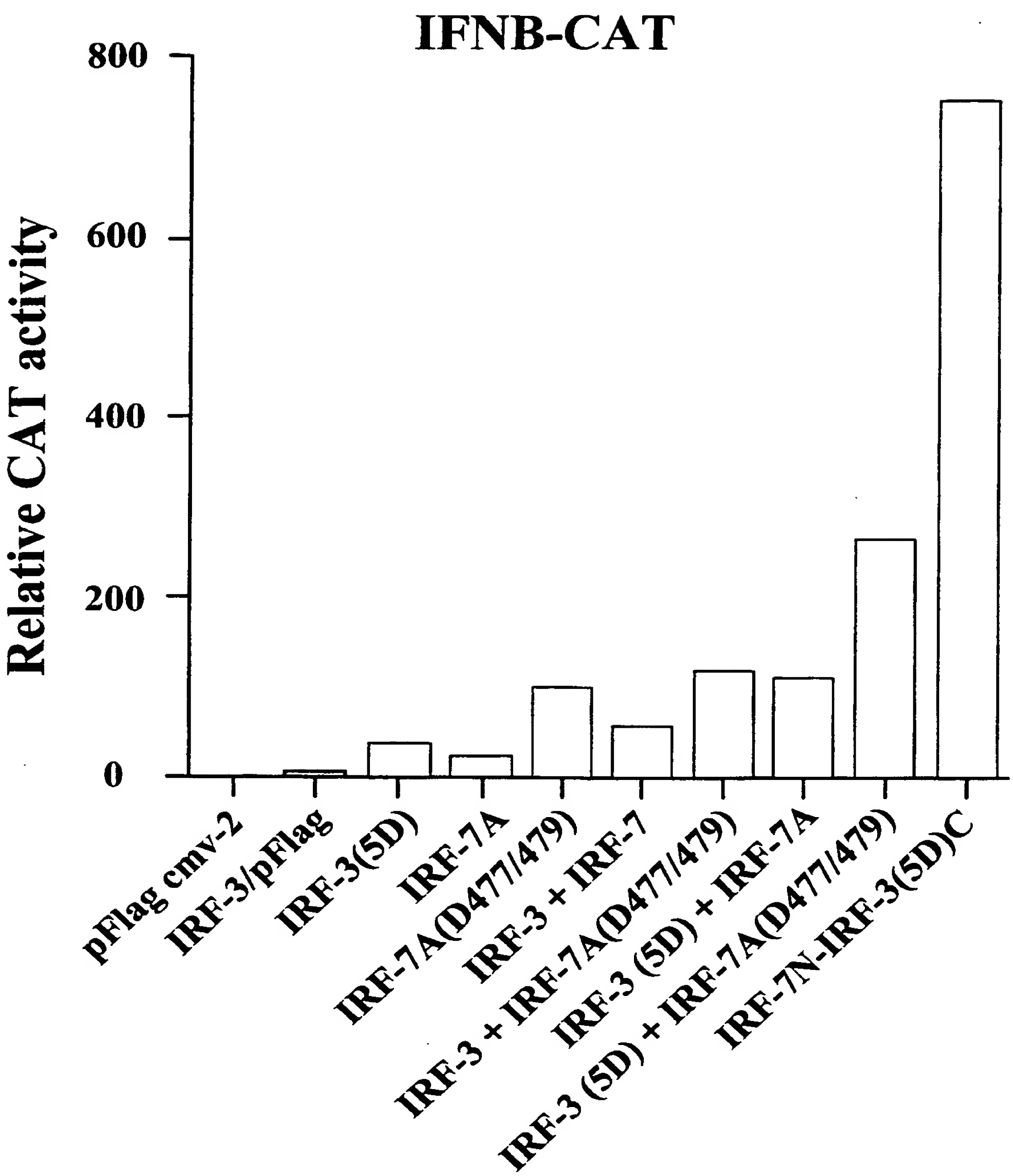
IFNB-CAT
800
600
400
200
0
Relative CAT activity
pFlag cmv-2
IRF-3/pFlag
IRF-3(5D)
IRF-7A
IRF-7A(D477/479)
IRF-3 + IRF-7
IRF-3 + IRF-7A(D477/479)
IRF-3 (5D) + IRF-7A
IRF-3 (5D) + IRF-7A(D477/479)
IRF-7N-IRF-3(5D)C

FIG. 14

SEQUENCE LISTING

<110> THE SIR MORTIMER B. DAVIS-JEWISH GENERAL HOSPITAL

<120> HIGHLY ACTIVE FORMS OF INTERFERON REGULATORY FACTOR PROTEINS

<130> IRF-3

<140>

<141>

<150> CA 2,234,588

<151> 1998-04-07

<160> 11

<170> PatentIn Ver. 2.0

<210> 1

<211> 1284

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<213> Homo sapiens

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<222> (1)..(1281)

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Met Gly Thr Pro Lys Pro Arg Ile Leu Pro Trp Leu Val Ser Gln Leu
  1               5                  10                  15

gac ctg ggg caa ctg gag ggc gtg gcc tgg gtg aac aag agc cgc acg    96
Asp Leu Gly Gln Leu Glu Gly Val Ala Trp Val Asn Lys Ser Arg Thr
             20                  25                  30

cgc ttc cgc atc cct tgg aag cac ggc cta cgg cag gat gca cag cag    144
Arg Phe Arg Ile Pro Trp Lys His Gly Leu Arg Gln Asp Ala Gln Gln
         35                  40                  45

gag gat ttc gga atc ttc cag gcc tgg gcc gag gcc act ggt gca tat    192
Glu Asp Phe Gly Ile Phe Gln Ala Trp Ala Glu Ala Thr Gly Ala Tyr
     50                  55                  60

gtt ccc ggg agg gat aag cca gac ctg cca acc tgg aag agg aat ttc    240
Val Pro Gly Arg Asp Lys Pro Asp Leu Pro Thr Trp Lys Arg Asn Phe
 65                  70                  75                  80
```

```
cgc tct gcc ctc aac cgc aaa gaa ggg ttg cgt tta gca gag gac cgg     288
Arg Ser Ala Leu Asn Arg Lys Glu Gly Leu Arg Leu Ala Glu Asp Arg
                 85                  90                  95

agc aag gac cct cac gac cca cat aaa atc tac gag ttt gtg aac tca     336
Ser Lys Asp Pro His Asp Pro His Lys Ile Tyr Glu Phe Val Asn Ser
            100                 105                 110

gga gtt ggg gac ttt tcc cag cca gac acc tct ccg gac acc aat ggt     384
Gly Val Gly Asp Phe Ser Gln Pro Asp Thr Ser Pro Asp Thr Asn Gly
        115                 120                 125

gga ggc agt act tct gat acc cag gaa gac att ctg gat gag tta ctg     432
Gly Gly Ser Thr Ser Asp Thr Gln Glu Asp Ile Leu Asp Glu Leu Leu
    130                 135                 140

ggt aac atg gtg ttg gcc cca ctc cca gat ccg gga ccc cca agc ctg     480
Gly Asn Met Val Leu Ala Pro Leu Pro Asp Pro Gly Pro Pro Ser Leu
145                 150                 155                 160

gct gta gcc cct gag ccc tgc cct cag ccc ctg cgg agc ccc agc ttg     528
Ala Val Ala Pro Glu Pro Cys Pro Gln Pro Leu Arg Ser Pro Ser Leu
                    165                 170                 175

gac aat ccc act ccc ttc cca aac ctg ggg ccc tct gag aac cca ctg     576
Asp Asn Pro Thr Pro Phe Pro Asn Leu Gly Pro Ser Glu Asn Pro Leu
                180                 185                 190

aag cgg ctg ttg gtg ccg ggg gaa gag tgg gag ttc gag gtg aca gcc     624
Lys Arg Leu Leu Val Pro Gly Glu Glu Trp Glu Phe Glu Val Thr Ala
            195                 200                 205

ttc tac cgg ggc cgc caa gtc ttc cag cag acc atc tcc tgc ccg gag     672
Phe Tyr Arg Gly Arg Gln Val Phe Gln Gln Thr Ile Ser Cys Pro Glu
        210                 215                 220

ggc ctg cgg ctg gtg ggg tcc gaa gtg gga gac agg acg ctg cct gga     720
Gly Leu Arg Leu Val Gly Ser Glu Val Gly Asp Arg Thr Leu Pro Gly
    225                 230                 235

tgg cca gtc aca ctg cca gac cct ggc atg tcc ctg aca gac agg gga     768
Trp Pro Val Thr Leu Pro Asp Pro Gly Met Ser Leu Thr Asp Arg Gly
240                 245                 250                 255

gtg atg agc tac gtg agg cat gtg ctg agc tgc ctg ggt ggg gga ctg     816
Val Met Ser Tyr Val Arg His Val Leu Ser Cys Leu Gly Gly Gly Leu
                260                 265                 270

gct ctc tgg cgg gcc ggg cag tgg ctc tgg gcc cag cgg ctg ggg cac     864
Ala Leu Trp Arg Ala Gly Gln Trp Leu Trp Ala Gln Arg Leu Gly His
            275                 280                 285

tgc cac aca tac tgg gca gtg agc gag gag ctg ctc ccc aac agc ggg     912
Cys His Thr Tyr Trp Ala Val Ser Glu Glu Leu Leu Pro Asn Ser Gly
        290                 295                 300

cat ggg cct gat ggc gag gtc ccc aag gac aag gaa gga ggc gtg ttt     960
His Gly Pro Asp Gly Glu Val Pro Lys Asp Lys Glu Gly Gly Val Phe
    305                 310                 315

gac ctg ggg ccc ttc att gta gat ctg att acc ttc acg gaa gga agc     1008
Asp Leu Gly Pro Phe Ile Val Asp Leu Ile Thr Phe Thr Glu Gly Ser
320                 325                 330                 335

gga cgc tca cca cgc tat gcc ctc tgg ttc tgt gtg ggg gag tca tgg     1056
Gly Arg Ser Pro Arg Tyr Ala Leu Trp Phe Cys Val Gly Glu Ser Trp
                340                 345                 350
```

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ccc cag gac cag ccg tgg acc aag agg ctc gtg atg gtc aag gtt gtg    1104
Pro Gln Asp Gln Pro Trp Thr Lys Arg Leu Val Met Val Lys Val Val
            355                 360                     365

ccc acg tgc ctc agg gcc ttg gta gaa atg gcc cgg gta ggg ggt gcc    1152
Pro Thr Cys Leu Arg Ala Leu Val Glu Met Ala Arg Val Gly Gly Ala
        370                 375                 380

tcc tcc ctg gag aat act gtg gac ctg cac att gac aac gac cac cca    1200
Ser Ser Leu Glu Asn Thr Val Asp Leu His Ile Asp Asn Asp His Pro
    385                 390                     395

ctc gac ctc gac gac gac cag tac aag gcc tac ctg cag gac ttg gtg    1248
Leu Asp Leu Asp Asp Asp Gln Tyr Lys Ala Tyr Leu Gln Asp Leu Val
400                 405                 410                     415

gag ggc atg gat ttc cag ggc cct ggg gag agc tga                    1284
Glu Gly Met Asp Phe Gln Gly Pro Gly Glu Ser
                420                 425
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<210> 2

<211> 427

<212> PRT

<213> Homo sapiens

<400> 2

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Met Gly Thr Pro Lys Pro Arg Ile Leu Pro Trp Leu Val Ser Gln Leu
 1               5                  10                  15

Asp Leu Gly Gln Leu Glu Gly Val Ala Trp Val Asn Lys Ser Arg Thr
            20                  25                  30

Arg Phe Arg Ile Pro Trp Lys His Gly Leu Arg Gln Asp Ala Gln Gln
            35                  40                  45

Glu Asp Phe Gly Ile Phe Gln Ala Trp Ala Glu Ala Thr Gly Ala Tyr
        50                  55                  60

Val Pro Gly Arg Asp Lys Pro Asp Leu Pro Thr Trp Lys Arg Asn Phe
    65                  70                  75

Arg Ser Ala Leu Asn Arg Lys Glu Gly Leu Arg Leu Ala Glu Asp Arg
80                  85                  90                      95

Ser Lys Asp Pro His Asp Pro His Lys Ile Tyr Glu Phe Val Asn Ser
                100                 105                 110

Gly Val Gly Asp Phe Ser Gln Pro Asp Thr Ser Pro Asp Thr Asn Gly
            115                 120                 125

Gly Gly Ser Thr Ser Asp Thr Gln Glu Asp Ile Leu Asp Glu Leu Leu
        130                 135                 140

Gly Asn Met Val Leu Ala Pro Leu Pro Asp Pro Gly Pro Pro Ser Leu
    145                 150                 155

Ala Val Ala Pro Glu Pro Cys Pro Gln Pro Leu Arg Ser Pro Ser Leu
160                 165                 170                     175

Asp Asn Pro Thr Pro Phe Pro Asn Leu Gly Pro Ser Glu Asn Pro Leu
                180                 185                 190
```

```
Lys Arg Leu Leu Val Pro Gly Glu Glu Trp Glu Phe Glu Val Thr Ala
            195                 200                 205

Phe Tyr Arg Gly Arg Gln Val Phe Gln Gln Thr Ile Ser Cys Pro Glu
        210                 215                 220

Gly Leu Arg Leu Val Gly Ser Glu Val Gly Asp Arg Thr Leu Pro Gly
    225                 230                 235

Trp Pro Val Thr Leu Pro Asp Pro Gly Met Ser Leu Thr Asp Arg Gly
240                 245                 250                     255

Val Met Ser Tyr Val Arg His Val Leu Ser Cys Leu Gly Gly Gly Leu
                260                 265                 270

Ala Leu Trp Arg Ala Gly Gln Trp Leu Trp Ala Gln Arg Leu Gly His
            275                 280                 285

Cys His Thr Tyr Trp Ala Val Ser Glu Glu Leu Leu Pro Asn Ser Gly
        290                 295                 300

His Gly Pro Asp Gly Glu Val Pro Lys Asp Lys Glu Gly Gly Val Phe
    305                 310                 315

Asp Leu Gly Pro Phe Ile Val Asp Leu Ile Thr Phe Thr Glu Gly Ser
320                 325                 330                     335

Gly Arg Ser Pro Arg Tyr Ala Leu Trp Phe Cys Val Gly Glu Ser Trp
                340                 345                 350

Pro Gln Asp Gln Pro Trp Thr Lys Arg Leu Val Met Val Lys Val Val
            355                 360                 365

Pro Thr Cys Leu Arg Ala Leu Val Glu Met Ala Arg Val Gly Gly Ala
        370                 375                 380

Ser Ser Leu Glu Asn Thr Val Asp Leu His Ile Asp Asn Asp His Pro
    385                 390                 395

Leu Asp Leu Asp Asp Asp Gln Tyr Lys Ala Tyr Leu Gln Asp Leu Val
400                 405                 410                     415

Glu Gly Met Asp Phe Gln Gly Pro Gly Glu Ser
                420                 425
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<210> 3

<211> 13

<212> PRT

<213> Homo sapiens

<400> 3

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Ile Ser Asn Ser His Pro Leu Ser Leu Thr Ser Asp Gln
  1               5                  10
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<210> 4

<211> 4

<212> PRT

<213> Homo sapiens

<400> 4

Gly Ala Ala Ala
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<210> 5

<211> 6

<212> PRT

<213> Homo sapiens

<400> 5

Gly Ala Ala Ala Asn Asn
1 5

<210> 6

<211> 15

<212> DNA

<213> Homo sapiens

<400> 6

ggaaaactga aaggg 15

<210> 7

<211> 30

<212> DNA

<213> Homo sapiens

<400> 7
gatcgggaaa gggaaaccga aactgaagcc 30

<210> 8

<211> 1512

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1509)

<400> 8

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Met Ala Leu Ala Pro Glu Arg Ala Ala Pro Arg Val Leu Phe Gly Glu
1 5 10 15

tgg ctc ctt gga gag atc agc agc ggc tgc tat gag ggg ctg cag tgg 96
Trp Leu Leu Gly Glu Ile Ser Ser Gly Cys Tyr Glu Gly Leu Gln Trp
20 25 30

```
ctg gac gag gcc cgc acc tgt ttc cgc gtg ccc tgg aag cac ttc gcg    144
Leu Asp Glu Ala Arg Thr Cys Phe Arg Val Pro Trp Lys His Phe Ala
         35                  40                  45

cgc aag gac ctg agc gag gcc gac gcg cgc atc ttc aag gcc tgg gct    192
Arg Lys Asp Leu Ser Glu Ala Asp Ala Arg Ile Phe Lys Ala Trp Ala
     50                  55                  60

gtg gcc cgc ggc agg tgg ccg cct agc agc agg gga ggt ggc ccg ccc    240
Val Ala Arg Gly Arg Trp Pro Pro Ser Ser Arg Gly Gly Gly Pro Pro
 65                  70                  75                  80

ccc gag gct gag act gcg gag cgc gcc ggc tgg aaa acc aac ttc cgc    288
Pro Glu Ala Glu Thr Ala Glu Arg Ala Gly Trp Lys Thr Asn Phe Arg
                 85                  90                  95
tgc gca ctg cgc agc acg cgt cgc ttc gtg atg ctg cgg gat aac tcg    336
Cys Ala Leu Arg Ser Thr Arg Arg Phe Val Met Leu Arg Asp Asn Ser
            100                 105                 110

ggg gac ccg gcc gac ccg cac aag gtg tac gcg ctc agc cgg gag ctg    384
Gly Asp Pro Ala Asp Pro His Lys Val Tyr Ala Leu Ser Arg Glu Leu
        115                 120                 125

tgc tgg cga gaa ggc cca ggc acg gac cag act gag gca gag gcc ccc    432
Cys Trp Arg Glu Gly Pro Gly Thr Asp Gln Thr Glu Ala Glu Ala Pro
    130                 135                 140

gca gct gtc cca cca cca cag ggt ggg ccc cca ggg cca ttc ttg gca    480
Ala Ala Val Pro Pro Pro Gln Gly Gly Pro Pro Gly Pro Phe Leu Ala
145                 150                 155                 160

cac aca cat gct gga ctc caa gcc cca ggc ccc ctc cct gcc cca gct    528
His Thr His Ala Gly Leu Gln Ala Pro Gly Pro Leu Pro Ala Pro Ala
                165                 170                 175

ggt gac aag ggg gac ctc ctg ctc cag gca gtg caa cag agc tgc ctg    576
Gly Asp Lys Gly Asp Leu Leu Leu Gln Ala Val Gln Gln Ser Cys Leu
            180                 185                 190

gca gac cat ctg ctg aca gcg tca tgg ggg gca gat cca gtc cca acc    624
Ala Asp His Leu Leu Thr Ala Ser Trp Gly Ala Asp Pro Val Pro Thr
        195                 200                 205

aag gct cct gga gag gga caa gaa ggg ctt ccc ctg act ggg gcc tgt    672
Lys Ala Pro Gly Glu Gly Gln Glu Gly Leu Pro Leu Thr Gly Ala Cys
    210                 215                 220

gct gga ggc cca ggg ctc cct gct ggg gag ctg tac ggg tgg gca gta    720
Ala Gly Gly Pro Gly Leu Pro Ala Gly Glu Leu Tyr Gly Trp Ala Val
225                 230                 235                 240

gag acg acc ccc agc ccc ggg ccc cag ccc gcg gca cta acg aca ggc    768
Glu Thr Thr Pro Ser Pro Gly Pro Gln Pro Ala Ala Leu Thr Thr Gly
                245                 250                 255

gag gcc gcg gcc cca gag tcc ccg cac cag gca gag ccg tac ctg tca    816
Glu Ala Ala Ala Pro Glu Ser Pro His Gln Ala Glu Pro Tyr Leu Ser
            260                 265                 270

ccc tcc cca agc gcc tgc acc gcg gtg caa gag ccc agc cca ggg gcg    864
Pro Ser Pro Ser Ala Cys Thr Ala Val Gln Glu Pro Ser Pro Gly Ala
        275                 280                 285

ctg gac gtg acc atc atg tac aag ggc cgc acg gtg ctg cag aag gtg    912
Leu Asp Val Thr Ile Met Tyr Lys Gly Arg Thr Val Leu Gln Lys Val
    290                 295                 300
```

gtg gga cac ccg agc tgc acg ttc cta tac ggc ccc cca gac cca gct 960
Val Gly His Pro Ser Cys Thr Phe Leu Tyr Gly Pro Pro Asp Pro Ala
305 310 315 320

gtc cgg gcc aca gac ccc cag cag gta gca ttc ccc agc cct gcc gag 1008
Val Arg Ala Thr Asp Pro Gln Gln Val Ala Phe Pro Ser Pro Ala Glu
325 330 335

ctc ccg gac cag aag cag ctg cgc tac acg gag gaa ctg ctg cgg cac 1056
Leu Pro Asp Gln Lys Gln Leu Arg Tyr Thr Glu Glu Leu Leu Arg His
340 345 350

gtg gcc cct ggg ttg cac ctg gag ctt cgg ggg cca cag ctg tgg gcc 1104
Val Ala Pro Gly Leu His Leu Glu Leu Arg Gly Pro Gln Leu Trp Ala
355 360 365

cgg cgc atg ggc aag tgc aag gtg tac tgg gag gtg ggc gga ccc cca 1152
Arg Arg Met Gly Lys Cys Lys Val Tyr Trp Glu Val Gly Gly Pro Pro
370 375 380

ggc tcc gcc agc ccc tcc acc cca gcc tgc ctg ctg cct cgg aac tgt 1200
Gly Ser Ala Ser Pro Ser Thr Pro Ala Cys Leu Leu Pro Arg Asn Cys
385 390 395 400

gac acc ccc atc ttc gac ttc aga gtc ttc ttc caa gag ctg gtg gaa 1248
Asp Thr Pro Ile Phe Asp Phe Arg Val Phe Phe Gln Glu Leu Val Glu
405 410 415

ttc cgg gca cgg cag cgc cgt ggc tcc cca cgc tat acc atc tac ctg 1296
Phe Arg Ala Arg Gln Arg Arg Gly Ser Pro Arg Tyr Thr Ile Tyr Leu
420 425 430

ggc ttc ggg cag gac ctg tca gct ggg agg ccc aag gag aag agc ctg 1344
Gly Phe Gly Gln Asp Leu Ser Ala Gly Arg Pro Lys Glu Lys Ser Leu
435 440 445

gtc ctg gtg aag ctg gaa ccc tgg ctg tgc cga gtg cac cta gag ggc 1392
Val Leu Val Lys Leu Glu Pro Trp Leu Cys Arg Val His Leu Glu Gly
450 455 460

acg cag cgt gag ggt gtg tct tcc ctg gat agc agc gac ctc gac ctc 1440
Thr Gln Arg Glu Gly Val Ser Ser Leu Asp Ser Ser Asp Leu Asp Leu
465 470 475 480

tgc ctg tcc agc gcc aac agc ctc tat gac gac atc gag tgc ttc ctt 1488
Cys Leu Ser Ser Ala Asn Ser Leu Tyr Asp Asp Ile Glu Cys Phe Leu
485 490 495

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<211> 503

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<213> Homo sapiens

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1 5 10 15

Trp Leu Leu Gly Glu Ile Ser Ser Gly Cys Tyr Glu Gly Leu Gln Trp
20 25 30

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Leu Asp Glu Ala Arg Thr Cys Phe Arg Val Pro Trp Lys His Phe Ala
         35                  40                  45

Arg Lys Asp Leu Ser Glu Ala Asp Ala Arg Ile Phe Lys Ala Trp Ala
     50                  55                  60

Val Ala Arg Gly Arg Trp Pro Pro Ser Ser Arg Gly Gly Gly Pro Pro
 65                  70                  75                  80

Pro Glu Ala Glu Thr Ala Glu Arg Ala Gly Trp Lys Thr Asn Phe Arg
                 85                  90                  95

Cys Ala Leu Arg Ser Thr Arg Arg Phe Val Met Leu Arg Asp Asn Ser
            100                 105                 110

Gly Asp Pro Ala Asp Pro His Lys Val Tyr Ala Leu Ser Arg Glu Leu
        115                 120                 125

Cys Trp Arg Glu Gly Pro Gly Thr Asp Gln Thr Glu Ala Glu Ala Pro
    130                 135                 140

Ala Ala Val Pro Pro Pro Gln Gly Gly Pro Pro Gly Pro Phe Leu Ala
145                 150                 155                 160

His Thr His Ala Gly Leu Gln Ala Pro Gly Pro Leu Pro Ala Pro Ala
                165                 170                 175

Gly Asp Lys Gly Asp Leu Leu Leu Gln Ala Val Gln Gln Ser Cys Leu
            180                 185                 190

Ala Asp His Leu Leu Thr Ala Ser Trp Gly Ala Asp Pro Val Pro Thr
        195                 200                 205

Lys Ala Pro Gly Glu Gly Gln Glu Gly Leu Pro Leu Thr Gly Ala Cys
    210                 215                 220

Ala Gly Gly Pro Gly Leu Pro Ala Gly Glu Leu Tyr Gly Trp Ala Val
225                 230                 235                 240

Glu Thr Thr Pro Ser Pro Gly Pro Gln Pro Ala Ala Leu Thr Thr Gly
                245                 250                 255

Glu Ala Ala Ala Pro Glu Ser Pro His Gln Ala Glu Pro Tyr Leu Ser
            260                 265                 270

Pro Ser Pro Ser Ala Cys Thr Ala Val Gln Glu Pro Ser Pro Gly Ala
        275                 280                 285

Leu Asp Val Thr Ile Met Tyr Lys Gly Arg Thr Val Leu Gln Lys Val
    290                 295                 300

Val Gly His Pro Ser Cys Thr Phe Leu Tyr Gly Pro Pro Asp Pro Ala
305                 310                 315                 320

Val Arg Ala Thr Asp Pro Gln Gln Val Ala Phe Pro Ser Pro Ala Glu
                325                 330                 335

Leu Pro Asp Gln Lys Gln Leu Arg Tyr Thr Glu Glu Leu Leu Arg His
            340                 345                 350

Val Ala Pro Gly Leu His Leu Glu Leu Arg Gly Pro Gln Leu Trp Ala
        355                 360                 365

Arg Arg Met Gly Lys Cys Lys Val Tyr Trp Glu Val Gly Gly Pro Pro
    370                 375                 380

Gly Ser Ala Ser Pro Ser Thr Pro Ala Cys Leu Leu Pro Arg Asn Cys
385                 390                 395                 400
```

```
Asp Thr Pro Ile Phe Asp Phe Arg Val Phe Phe Gln Glu Leu Val Glu
                405                 410                 415

Phe Arg Ala Arg Gln Arg Arg Gly Ser Pro Arg Tyr Thr Ile Tyr Leu
            420                 425                 430

Gly Phe Gly Gln Asp Leu Ser Ala Gly Arg Pro Lys Glu Lys Ser Leu
        435                 440                 445

Val Leu Val Lys Leu Glu Pro Trp Leu Cys Arg Val His Leu Glu Gly
    450                 455                 460

Thr Gln Arg Glu Gly Val Ser Ser Leu Asp Ser Ser Asp Leu Asp Leu
465                 470                 475                 480

Cys Leu Ser Ser Ala Asn Ser Leu Tyr Asp Asp Ile Glu Cys Phe Leu
                485                 490                 495

Met Glu Leu Glu Gln Pro Ala
            500
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<210> 10

<211> 1629

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1626)

<400> 10

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atg gcc ttg gct cct gag agg gca gcc cca cgc gtg ctg ttc gga gag      48
Met Ala Leu Ala Pro Glu Arg Ala Ala Pro Arg Val Leu Phe Gly Glu
  1               5                  10                  15

tgg ctc ctt gga gag atc agc agc ggc tgc tat gag ggg ctg cag tgg      96
Trp Leu Leu Gly Glu Ile Ser Ser Gly Cys Tyr Glu Gly Leu Gln Trp
             20                  25                  30

ctg gac gag gcc cgc acc tgt ttc cgc gtg ccc tgg aag cac ttc gcg     144
Leu Asp Glu Ala Arg Thr Cys Phe Arg Val Pro Trp Lys His Phe Ala
         35                  40                  45

cgc aag gac ctg agc gag gcc gac gcg cgc atc ttc aag gcc tgg gct     192
Arg Lys Asp Leu Ser Glu Ala Asp Ala Arg Ile Phe Lys Ala Trp Ala
     50                  55                  60

gtg gcc cgc ggc agg tgg ccg cct agc agc agg gga ggt ggc ccg ccc     240
Val Ala Arg Gly Arg Trp Pro Pro Ser Ser Arg Gly Gly Gly Pro Pro
 65                  70                  75                  80

ccc gag gct gag act gcg gag cgc gcc ggc tgg aaa acc aac ttc cgc     288
Pro Glu Ala Glu Thr Ala Glu Arg Ala Gly Trp Lys Thr Asn Phe Arg
                 85                  90                  95

tgc gca ctg cgc agc acg cgt cgc ttc gtg atg ctg cgg gat aac tcg     336
Cys Ala Leu Arg Ser Thr Arg Arg Phe Val Met Leu Arg Asp Asn Ser
            100                 105                 110
```

ggg gac ccg gcc gac ccg cac aag gtg tac gcg ctc agc cgg gag ctg 384
Gly Asp Pro Ala Asp Pro His Lys Val Tyr Ala Leu Ser Arg Glu Leu
115 120 125

tgc tgg cga gaa ggc cca ggc acg gac cag act gag gca gag gcc ccc 432
Cys Trp Arg Glu Gly Pro Gly Thr Asp Gln Thr Glu Ala Glu Ala Pro
130 135 140

gca gct gtc cca cca cca cag ggt ggg ccc cca ggg cca ttc ttg gca 480
Ala Ala Val Pro Pro Pro Gln Gly Gly Pro Pro Gly Pro Phe Leu Ala
145 150 155 160

cac aca cat gct gga ctc caa gcc cca ggc ccc ctc cct gcc cca gct 528
His Thr His Ala Gly Leu Gln Ala Pro Gly Pro Leu Pro Ala Pro Ala
165 170 175

ggt gac aag ggg gac ctc ctg ctc cag gca gtg caa cag agc tgc ctg 576
Gly Asp Lys Gly Asp Leu Leu Leu Gln Ala Val Gln Gln Ser Cys Leu
180 185 190

gca gac cat ctg ctg aca gcg tca tgg ggg gca gat cca gtc cca acc 624
Ala Asp His Leu Leu Thr Ala Ser Trp Gly Ala Asp Pro Val Pro Thr
195 200 205

aag gct cct gga gag gga caa gaa ggg ctt ccc ctg act ggg gcc tgt 672
Lys Ala Pro Gly Glu Gly Gln Glu Gly Leu Pro Leu Thr Gly Ala Cys
210 215 220

gct gga ggc cca ggg ctc cct gct ggg gag ctg tac ggg tgg gca gta 720
Ala Gly Gly Pro Gly Leu Pro Ala Gly Glu Leu Tyr Gly Trp Ala Val
225 230 235 240

gag acg acc ccc agc ccc act tct gat acc cag gaa gac att ctg gat 768
Glu Thr Thr Pro Ser Pro Thr Ser Asp Thr Gln Glu Asp Ile Leu Asp
245 250 255

gag tta ctg ggt aac atg gtg ttg gcc cca ctc cca gat ccg gga ccc 816
Glu Leu Leu Gly Asn Met Val Leu Ala Pro Leu Pro Asp Pro Gly Pro
260 265 270

cca agc ctg gct gta gcc cct gag ccc tgc cct cag ccc ctg cgg agc 864
Pro Ser Leu Ala Val Ala Pro Glu Pro Cys Pro Gln Pro Leu Arg Ser
275 280 285

ccc agc ttg gac aat ccc act ccc ttc cca aac ctg ggg ccc tct gag 912
Pro Ser Leu Asp Asn Pro Thr Pro Phe Pro Asn Leu Gly Pro Ser Glu
290 295 300

aac cca ctg aag cgg ctg ttg gtg ccg ggg gaa gag tgg gag ttc gag 960
Asn Pro Leu Lys Arg Leu Leu Val Pro Gly Glu Glu Trp Glu Phe Glu
305 310 315 320

gtg aca gcc ttc tac cgg ggc cgc caa gtc ttc cag cag acc atc tcc 1008
Val Thr Ala Phe Tyr Arg Gly Arg Gln Val Phe Gln Gln Thr Ile Ser
325 330 335

tgc ccg gag ggc ctg cgg ctg gtg ggg tcc gaa gtg gga gac agg acg 1056
Cys Pro Glu Gly Leu Arg Leu Val Gly Ser Glu Val Gly Asp Arg Thr
340 345 350

ctg cct gga tgg cca gtc aca ctg cca gac cct ggc atg tcc ctg aca 1104
Leu Pro Gly Trp Pro Val Thr Leu Pro Asp Pro Gly Met Ser Leu Thr
355 360 365

gac agg gga gtg atg agc tac gtg agg cat gtg ctg agc tgc ctg ggt 1152
Asp Arg Gly Val Met Ser Tyr Val Arg His Val Leu Ser Cys Leu Gly
370 375 380

ggg gga ctg gct ctc tgg cgg gcc ggg cag tgg ctc tgg gcc cag cgg 1200
Gly Gly Leu Ala Leu Trp Arg Ala Gly Gln Trp Leu Trp Ala Gln Arg
385 390 395 400

ctg ggg cac tgc cac aca tac tgg gca gtg agc gag gag ctg ctc ccc 1248
Leu Gly His Cys His Thr Tyr Trp Ala Val Ser Glu Glu Leu Leu Pro
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aac agc ggg cat ggg cct gat ggc gag gtc ccc aag gac aag gaa gga 1296
Asn Ser Gly His Gly Pro Asp Gly Glu Val Pro Lys Asp Lys Glu Gly
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ggc gtg ttt gac ctg ggg ccc ttc att gta gat ctg att acc ttc acg 1344
Gly Val Phe Asp Leu Gly Pro Phe Ile Val Asp Leu Ile Thr Phe Thr
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gaa gga agc gga cgc tca cca cgc tat gcc ctc tgg ttc tgt gtg ggg 1392
Glu Gly Ser Gly Arg Ser Pro Arg Tyr Ala Leu Trp Phe Cys Val Gly
450 455 460

gag tca tgg ccc cag gac cag ccg tgg acc aag agg ctc gtg atg gtc 1440
Glu Ser Trp Pro Gln Asp Gln Pro Trp Thr Lys Arg Leu Val Met Val
465 470 475 480

aag gtt gtg ccc acg tgc ctc agg gcc ttg gta gaa atg gcc cgg gta 1488
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485 490 495

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Gly Gly Ala Ser Ser Leu Glu Asn Thr Val Asp Leu His Ile Asp Asn
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gac cac cca ctc gac ctc gac gac gac cag tac aag gcc tac ctg cag 1584
Asp His Pro Leu Asp Leu Asp Asp Asp Gln Tyr Lys Ala Tyr Leu Gln
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<211> 542

<212> PRT

<213> Homo sapiens

<400> 11

Met Ala Leu Ala Pro Glu Arg Ala Ala Pro Arg Val Leu Phe Gly Glu
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Trp Leu Leu Gly Glu Ile Ser Ser Gly Cys Tyr Glu Gly Leu Gln Trp
20 25 30

Leu Asp Glu Ala Arg Thr Cys Phe Arg Val Pro Trp Lys His Phe Ala
35 40 45

Arg Lys Asp Leu Ser Glu Ala Asp Ala Arg Ile Phe Lys Ala Trp Ala
50 55 60

Val Ala Arg Gly Arg Trp Pro Pro Ser Ser Arg Gly Gly Gly Pro Pro
65 70 75 80

Pro Glu Ala Glu Thr Ala Glu Arg Ala Gly Trp Lys Thr Asn Phe Arg
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Cys Ala Leu Arg Ser Thr Arg Arg Phe Val Met Leu Arg Asp Asn Ser
            100                 105                 110

Gly Asp Pro Ala Asp Pro His Lys Val Tyr Ala Leu Ser Arg Glu Leu
        115                 120                 125

Cys Trp Arg Glu Gly Pro Gly Thr Asp Gln Thr Glu Ala Glu Ala Pro
    130                 135                 140

Ala Ala Val Pro Pro Pro Gln Gly Gly Pro Pro Gly Pro Phe Leu Ala
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His Thr His Ala Gly Leu Gln Ala Pro Gly Pro Leu Pro Ala Pro Ala
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Gly Asp Lys Gly Asp Leu Leu Leu Gln Ala Val Gln Gln Ser Cys Leu
            180                 185                 190

Ala Asp His Leu Leu Thr Ala Ser Trp Gly Ala Asp Pro Val Pro Thr
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Lys Ala Pro Gly Glu Gly Gln Glu Gly Leu Pro Leu Thr Gly Ala Cys
    210                 215                 220

Ala Gly Gly Pro Gly Leu Pro Ala Gly Glu Leu Tyr Gly Trp Ala Val
225                 230                 235                 240

Glu Thr Thr Pro Ser Pro Thr Ser Asp Thr Gln Glu Asp Ile Leu Asp
                245                 250                 255

Glu Leu Leu Gly Asn Met Val Leu Ala Pro Leu Pro Asp Pro Gly Pro
            260                 265                 270

Pro Ser Leu Ala Val Ala Pro Glu Pro Cys Pro Gln Pro Leu Arg Ser
        275                 280                 285

Pro Ser Leu Asp Asn Pro Thr Pro Phe Pro Asn Leu Gly Pro Ser Glu
    290                 295                 300

Asn Pro Leu Lys Arg Leu Leu Val Pro Gly Glu Glu Trp Glu Phe Glu
305                 310                 315                 320

Val Thr Ala Phe Tyr Arg Gly Arg Gln Val Phe Gln Gln Thr Ile Ser
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Cys Pro Glu Gly Leu Arg Leu Val Gly Ser Glu Val Gly Asp Arg Thr
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        355                 360                 365

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    370                 375                 380

Gly Gly Leu Ala Leu Trp Arg Ala Gly Gln Trp Leu Trp Ala Gln Arg
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Leu Gly His Cys His Thr Tyr Trp Ala Val Ser Glu Glu Leu Leu Pro
                405                 410                 415

Asn Ser Gly His Gly Pro Asp Gly Glu Val Pro Lys Asp Lys Glu Gly
            420                 425                 430

Gly Val Phe Asp Leu Gly Pro Phe Ile Val Asp Leu Ile Thr Phe Thr
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Asp His Pro Leu Asp Leu Asp Asp Asp Gln Tyr Lys Ala Tyr Leu Gln
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Asp Leu Val Glu Gly Met Asp Phe Gln Gly Pro Gly Glu Ser
    530                 535                 540
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/00314

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 A61K38/17 A61K48/00 C07K19/00
C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MITSUTOSHI YONEYAMA ET AL: "Direct triggering of the type I interferon systemby virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300" EMBO JOURNAL., vol. 17, no. 4, 16 February 1998 (1998-02-16), pages 1087-1095, XP002110452 OXFORD UNIVERSITY PRESS, SURREY., GB ISSN: 0261-4189 page 1089, right-hand column, paragraph 2 - page 1090 page 1089, left-hand column, paragraph 3 - right-hand column, paragraph 1 figure 4A ---	1,3,15, 16,21,22

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
2 August 1999	17/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Cornec, N

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WEI-CHUN AU ET AL: "Identification of a member of the interferon regulatory factor family that binds to the interferon-stimulated response element and activates expression of interferon-induced genes " PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 92, December 1995 (1995-12), pages 11657-11661, XP000490487 NATIONAL ACADEMY OF SCIENCE. WASHINGTON., US ISSN: 0027-8424 cited in the application the whole document	15,16,18
A		21,22

X	L. ZHANG ET AL: EMBL DATABASE ENTRY HSU53830, ACCESSION NUMBER U53830, 19 May 1997 (1997-05-19), XP002110966 cited in the application abstract -& L. ZHANG ET AL: "IRF-7, a new Interferon Regulatory Factor associated with Epstein -Barr virus latency" MOLECULAR AND CELLULAR BIOLOGY., vol. 17, no. 10, October 1997 (1997-10), pages 5748-5737, XP002110967 AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON., US ISSN: 0270-7306	15,17,18

X	A. GROSSMAN ET AL: "Characterization of IRF-7, a novel Interferon Regulatory Factor " EMBL DATABASE ENTRY HSU73036, ACCESSION NUMBER U73036 , 21 October 1996 (1996-10-21), XP002110973 cited in the application abstract & UNPUBLISHED,	15,17,18

P,X	R. LIN ET AL: "Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome mediated degradation" MOLECULAR AND CELLULAR BIOLOGY., vol. 18, no. 5, May 1998 (1998-05), pages 2986-2996, XP002110454 AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON., US ISSN: 0270-7306 the whole document	1-9,15, 16,19, 21,22

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 99/00314

B x I Observations where c rtain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 21-22 (as far as they concern an in vivo method) and claims 23-34 are directed to a method of treatment of the human/animal body (rule 39.1 (IV) PCT, the search been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/00314

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	R. LIN ET AL: "Essential role of interferon regulatory factor 3 in direct activation of RANTES chemokine transcription" MOLECULAR AND CELLULAR BIOLOGY., vol. 19, no. 2, February 1999 (1999-02), pages 959-966, XP002110455 AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON., US ISSN: 0270-7306 the whole document ---	1-9,15, 16,19-22
T	R. LIN ET AL: "Structural and functional analysis of interferon regulatory factor-3: Localization of the Transactivation and autoinhibitory domains" MOLECULAR AND CELLULAR BIOLOGY., vol. 19, no. 4, April 1999 (1999-04), pages 2465-2474, XP002110456 AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON., US ISSN: 0270-7306 -----	

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/47, A61K 38/17, 48/00, C07K 19/00, C12N 15/62

A1

(11) International Publication Number: WO 99/51737

(43) International Publication Date: 14 October 1999 (14.10.99)

(21) International Application Number: PCT/CA99/00314

(22) International Filing Date: 7 April 1999 (07.04.99)

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2,234,588 7 April 1998 (07.04.98) CA

(71) Applicant *(for all designated States except US)*: THE SIR MORTIMER B. DAVIS–JEWISH GENERAL HOSPITAL [CA/CA]; 3755 chemin de la Cote–Sainte–Catherine, Montreal, Quebec H3T 1E2 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HISCOTT, John [CA/CA]; 132 Sheraton Drive, Montreal West, Quebec H4X 1N4 (CA). LIN, Rongtuan [CA/CA]; Apartment 17, 4455 Dupuis, Montreal, Quebec H3T 1E7 (CA).

(74) Agents: MORROW, Joy, D. et al.; Smart & Biggar, 900–55 Metcalfe Street, Station D, P.O. Box 2999, Ottawa, Ontario K1P 5Y6 (CA).

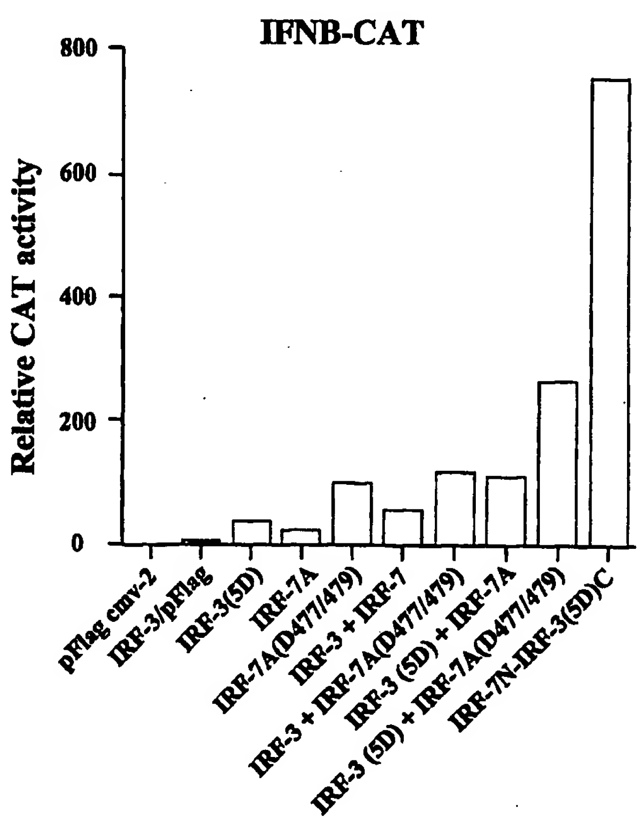
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HIGHLY ACTIVE FORMS OF INTERFERON REGULATORY FACTOR PROTEINS

(57) Abstract

The present invention relates to IRF proteins that have been modified in the carboxy–terminus domain (transactivation domain) by modification of serine and/or threonine sites. Modification may be achieved by phosphorylation of serine and/or threonine, or by replacement of serine and/or threonine residues with residues having acidic side–chains, preferably carboxylic acid–containing side–chains, such as aspartic acid or glutamic acid residues. Such modified proteins may be mutants of IRF–3 and IRF–7, including chimeric proteins having portions of both IRF–3 and IRF–7, and post–translationally modified (phosphorylated) IRF–3 protein, the phosphorylation being induced by Sendai virus infection. More specifically, the present invention provides a modified interferon regulatory factor (IRF) protein, the protein comprising at least one modified serine or threonine phosphoacceptor site in the carboxy–terminus domain, preferably wherein cytokine gene activation by the modified IRF is increased relative to cytokine gene activation by a corresponding wild type IRF protein. The invention also provides for pharmaceutical compositions containing IRF protein, and uses of the protein, nucleotide sequence encoding it, and pharmaceutical compositions containing it.



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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/00314

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 A61K38/17 A61K48/00 C07K19/00
C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 1999	17/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Cornec, N

5

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X A	WEI-CHUN AU ET AL: "Identification of a member of the interferon regulatory factor family that binds to the interferon-stimulated response element and activates expression of interferon-induced genes " PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 92, December 1995 (1995-12), pages 11657-11661, XP000490487 NATIONAL ACADEMY OF SCIENCE. WASHINGTON., US ISSN: 0027-8424 cited in the application the whole document ---	15,16,18 21,22
X	L. ZHANG ET AL: EMBL DATABASE ENTRY HSU53830, ACCESSION NUMBER U53830, 19 May 1997 (1997-05-19), XP002110966 cited in the application abstract -& L. ZHANG ET AL: "IRF-7, a new Interferon Regulatory Factor associated with Epstein -Barr virus latency" MOLECULAR AND CELLULAR BIOLOGY., vol. 17, no. 10, October 1997 (1997-10), pages 5748-5737, XP002110967 AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON., US ISSN: 0270-7306 ---	15,17,18
X	A. GROSSMAN ET AL: "Characterization of IRF-7, a novel Interferon Regulatory Factor " EMBL DATABASE ENTRY HSU73036, ACCESSION NUMBER U73036 , 21 October 1996 (1996-10-21), XP002110973 cited in the application abstract & UNPUBLISHED, ---	15,17,18
P,X	R. LIN ET AL: "Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome mediated degradation" MOLECULAR AND CELLULAR BIOLOGY., vol. 18, no. 5, May 1998 (1998-05), pages 2986-2996, XP002110454 AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON., US ISSN: 0270-7306 the whole document --- -/--	1-9,15, 16,19, 21,22

5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 99/00314

Box I Observations wher certain claims were found unsearchable (Continuati n of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:
   Remark: Although claims 21-22 (as far as they concern an in vivo method) and claims 23-34 are directed to a method of treatment of the human/animal body (rule 39.1 (IV) PCT, the search been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	R. LIN ET AL: "Essential role of interferon regulatory factor 3 in direct activation of RANTES chemokine transcription" MOLECULAR AND CELLULAR BIOLOGY., vol. 19, no. 2, February 1999 (1999-02), pages 959-966, XP002110455 AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON., US ISSN: 0270-7306 the whole document ---	1-9,15, 16,19-22
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